Annexe 2.2 :

Annex 2.2:

Digestibilité des mannoprotéines et des parois de levures

Digestibility of mannoproteins and cell walls

Adrian J. Frangne R. et Potus J. (1996)
les parois de levures alimentaires et leurs incidences nutritionnelles.
Med. Et Nutr., 37(4) :167-170.

Moine-Ledoux V. (2003)
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Méd. et Nut. — 1996. — T. 32. — N° 4.

LES PAROIS DES LEVURES ALIMENTAIRES ET LEURS INCIDENCES NUTRITIONNELLES.

J. ADRIAN, R. FRANGNE, J. POTUS*

RÉSUMÉ

Les enveloppes (paroi et membrane) des levures alimentaires renferment une large proportion de glucides et la quasi-totalité des lipides contenus dans ces produits.
Apparemment, les lipides sont fortement imbriqués dans la structure pariétale ; leur digestibilité n'est que 35 %.
Les deux tiers des glucides de levure constituent une fibre alimentaire, presque totalement dégradée par la flore intestinale. Cette fibre ne présente pas d'effets négatifs sur le plan de la digestion.
La valeur énergétique des levures alimentaires peut être estimée à 285 kcal/100 g ou 1 200 kJ.

Mots-clés : Fibre alimentaire - Glycane - Levure - Lipide.

ABSTRACT

Shell of food yeasts and their nutritional impacts.

The shell (wall and membrane) of food yeast contains a large proportion of carbohydrates and almost all of lipids held in this product. Lipids, strongly imbricated in the wall organization, have a low digestibility (35 %).
Two thirds of yeast carbohydrates constitute a fiber that is completely degraded by the intestinal flora. This fiber does not reveal negative effects in the digestibility phenomena.
The energy value of food yeasts can be estimate at 285 kCal/100 g or 1 200 kJ.

Key-words : Food fiber - Glycan - Lipid - Yeast.

Les levures sèches entrent depuis plusieurs décennies dans l'alimentation, tant humaine qu'animale, en raison de leurs propriétés nutritionnelles très caractéristiques : abondance des protéines, richesse en lysine, teneurs très élevées en vitamines du groupe B ; elles sont également employées pour leur potentiel organoleptique.

Les levures sont utilisées sous deux formes principales, soit à l'état tué et sec après avoir subi un choc thermique suffisant pour que leur contenu nutritionnel atteigne sa pleine efficacité, soit sous forme d'autolysat ou extrait de levure. Dans ce cas, la levure subit une autolyse qui solubilise ses éléments cytoplasmiques tandis que la paroi cellulaire — au sens large — demeure insoluble. Une centrifugation sépare les deux fractions de l'autolyse, le résidu non solubilisé comprend essentiellement la paroi et la membrane qui lui est associée. Nous le désignerons sous le nom générique d'enveloppes.

Cette fraction, riche en glucides, retient moins l'attention car les polyosides qu'elle renferme ont une valeur alimentaire apparemment très médiocre en raison de leur nature atypique. Ils se distinguent tout à fait du glycogène, glucide de réserve accumulé par la levure vivante en fonction du contexte physiologique dans lequel elle se trouve. Ce constituant se dégrade rapidement au cours des opérations technologiques de récolte et de séchage.

Une fois tuée, la levure ne renferme essentiellement que des glucides complexes de soutien qui forment l'architecture de la paroi cellulaire. Ils sont l'équivalent des éléments ligno-cellulosiques des végétaux supérieurs, mais leur nature en est tout à fait différente.

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Nous voudrions les décrire rapidement et situer leur place sur le plan nutritionnel ; leur rôle en alimentation ne peut être négligé étant donné qu'ils représentent entre 25 et 35 % de la matière sèche d'une levure alimentaire.

LOCALISATION ET NATURE DES GLUCIDES DE LA LEVURE

En dehors du glycogène, les glucides de la levure sont localisés dans les enveloppes de la cellule, qui représentent environ 20 % de la matière sèche de la levure de boulangerie. Ces enveloppes se composent d'une paroi externe et d'une membrane cytoplasmique, chaque élément étant lui-même de nature hétérogène, constitué de diverses couches de composition différente : la paroi est riche en polymères glucidiques tandis que la membrane est de nature lipo-protidique (tableau I).

En g/100 g	Paroi	Membrane
Glycanes	76,5 (60,0-84,5)	16,5 (3,2-30,8)
Protéines	9,2 (6,7-13,0)	39,5 (26,6-49,3)
Lipides	5,7 (2,0- 8,5)	40,5 (33,3-45,6)

Tableau I : Caractéristiques globales des enveloppes de levure (d'après SUOMALAINEN *et al.*, 1973).

Sur le plan chimique, les glucides pariétaux de la levure sont à la fois complexes et atypiques, ainsi qu'il ressort d'une des analyses les plus détaillées (tableau II). L'originalité des glucides de la levure réside dans les principales caractéristiques suivantes :

- la cellulose, les hémicelluloses, les pectines ainsi que la lignine ne sont pas identifiées parmi les glucides de la levure, alors que ce sont les composants classiques des parois des végétaux supérieurs ;
- les glucides prépondérants sont des hétéropolymères composés d'hexoses : glucose, galactose et mannose. Ils représentent plus de 80 % des glucides totaux ou 23 g/100 g de levure ;
- des oligosaccharides et un disaccharide spécifique, le tréhalose, sont à des taux minimes mais variables selon les conditions de culture ;

	En g/100 g	En % du total
Glucogalactanes	9,5	34,4
Galactomannanes	8,0	28,9
Glucomannanes	5,5	19,9
Tréhalose	0,25	0,9
Oligosaccharides	0,6	2,2
Glycogène	3,6	13,0
Sucres réducteurs	0,25	0,9

Tableau II : Nature des glucides d'une levure de lactosérum (culture aérobie) (LIEBRECHT, 1969).

- quelques éléments dont la valeur alimentaire est bien établie représentent de l'ordre de 15 % des glucides totaux, soit 4 g/100 g dans l'analyse du tableau II. Il s'agit essentiellement du glycogène qu'accompagne une petite quantité de sucres réducteurs.

Au vu de cette analyse, la levure contient peu d'éléments entrant dans la catégorie des glucides digestibles, c'est-à-dire hydrolysables par les amylases et les disaccharidases de l'appareil digestif (BERNIER *et al.*, 1988). Pour la plupart, ils répondent au concept de la fibre alimentaire, dans la mesure où ils sont peu ou pas absorbables au niveau de l'intestin grêle, mais dégradables par la flore colique.

Cette conclusion est confortée par les techniques récentes d'analyse. La mesure de la fibre selon la méthode A.O.A.C. (1990) consiste à soumettre un échantillon délipidé à l'action d'une amylase, puis d'une protéase et à peser le résidu final défalqué de ses éléments minéraux. Cette technique appliquée à une levure de boulangerie aboutit à une valeur de 26,5 g de fibre/100 g de levure sèche. Ce résultat est à rapprocher des données du tableau II, dans lequel les glucides présumés indigestibles dépassent 23 g/100 g.

ANALYSE DES ENVELOPPES DE LEVURE

Les enveloppes globales de levure (membrane et paroi) forment une structure fortement organisée à tel point que leur analyse chimique exige des procédures particulières pour atteindre un résultat satisfaisant. D'une part une rupture physique se révèle nécessaire ; elle peut être réalisée par une homogénéisation sous forte pression. De l'autre, le dosage des glucides et des lipides requiert des modalités appropriées. C'est ainsi qu'une hydrolyse sulfurique (2,5 % v/v) est indispensable pour hydrolyser les glycanes les plus résistants. De même, une extraction totale des lipides ne peut être obtenue qu'en faisant appel à des pratiques inhabituelles : par exemple, trois extractions successives à l'aide du butanol saturé, pendant 36 h à la température ambiante plus un chauffage à 70° C pendant 2 ou 3 h (ADRIAN et FRANGNE, 1976).

Avec ces procédés, on arrive à une analyse complète des enveloppes, à la condition qu'elles aient été au préalable physiquement rompues par un traitement sous pression (tableau III). En absence de cette phase préliminaire, les modalités d'extraction — même sévères — ne peuvent doser la totalité des composants contenus dans ces enveloppes cellulaires d'une levure de boulangerie (ADRIAN et FRANGNE, 1976).

Ces résultats mettent en lumière deux faits importants :

- les lipides apparaissent fortement imbriqués dans l'édifice glucidique et/ou protidique, la rupture

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En g/100 g	Enveloppe atomisée	Enveloppe homogénéisée puis atomisée
Eau	5,9	3,2
Cendres totales	2,9	3,7
Protéines brutes (N X 6,25)	20,1	19,7
Lipides totaux	23,6	30,3
Glucides totaux :		
- hydrolyse de 3 h	31,7	35,5
- hydrolyse de 10 h	34,9	40,5
Total dosé	87,4	97,4

Tableau III : Analyse chimique des enveloppes de levure en fonction de leur préparation et des modalités analytiques (ADRIAN et FRANGNE, 1976).

physique de la structure facilitant leur extraction. On peut penser à une association complexe entre la membrane lipo-protidique et la paroi glucidique ;
- les glucides des enveloppes sont composés essentiellement d'oses réducteurs, étant donné que le dosage basé sur le pouvoir réducteur (BERTRAND-SOMOGYI) et celui utilisant la réactivité avec un phénol en milieu sulfurique (DUBOIS) fournissent des valeurs proches l'une de l'autre. Comme en ce qui concerne les lipides, une déstructuration physique préalable des enveloppes est indispensable pour obtenir une hydrolyse totale des glycanes : une hydrolyse de 10 h sans étape physique préliminaire n'aboutit qu'à un résultat incomplet (tableau III).

On peut dès lors s'interroger sur la digestibilité des enveloppes et sur les répercussions qu'elles peuvent entraîner sur le plan nutritionnel.

DIGESTIBILITÉ DES ENVELOPPES DE LEVURE

Elle a été mesurée chez le rat recevant une ration témoin ou un régime contenant 20 % d'enveloppes de levure, homogénéisées ou non sous pression puis séchées. Le traitement physique de désintégration n'offre pas une amélioration significative de la digestibilité, contrairement à ce qui a été observé au moment des analyses chimiques.

Dans un but de simplification, les résultats sont présentés sous forme d'une moyenne pondérée des valeurs enregistrées avec les deux types d'enveloppes (tableau IV).

Pourcentage de digestibilité	Régime témoin (2 % de cellulose)	Régime avec 20 % d'enveloppes
Globale	96,18	92,07
Azotée	98,75	96,92
Lipidique	82,50	34,41
Glucidique	99,40	89,10

Tableau IV : Digestibilité des enveloppes de levure (ADRIAN et FRANGNE, 1976).

Les principales conclusions qui s'en dégagent sont :
- une digestibilité globale élevée des enveloppes (92 %), à peine inférieure à celle de la ration témoin ;
- une digestibilité remarquable de l'azote, proche de 97 %, indiquant que les protéases du tube digestif ont pu agir facilement sur les protéines incluses dans l'édifice pariétal ;
- à l'inverse, une digestibilité lipidique de 35 %, très faible, signifiant que les lipides n'ont pu être libérés et hydrolysés suffisamment tôt au cours du transit digestif pour pouvoir être absorbés par l'intestin grêle ;
- enfin, une digestibilité glucidique de 89 %, c'est-à-dire élevée en apparence. Cette valeur démontre que si les glucides ne sont pas digestibles au sens physiologique du terme, ils sont aisément utilisables par la flore intestinale. On peut avancer que la fraction non dégradée doit correspondre à celle qui est le plus intimement liée aux éléments lipidiques et, par conséquent, moins accessible aux glycanases d'origine microbiologique.

La fermentescibilité des glucides est nettement établie au vu de la biométrie du caecum : dans le lot témoin le poids de l'organe est de 0,76 g/100 g vif et chez les animaux consommant 20 % d'enveloppes il atteint 3,27 g, soit une hypertrophie d'environ 430 %.

INCIDENCES DES ENVELOPPES DE LEVURE SUR LE PLAN NUTRITIONNEL

Rappelons d'abord qu'à l'état natif, la structure physique de ces enveloppes constitue un obstacle à l'efficacité nutritionnelle du contenu cellulaire. C'est pourquoi, pendant leur séchage les levures doivent supporter un choc thermique d'intensité suffisante pour déstabiliser l'organisation de la paroi et permettre la pleine utilisation du potentiel azoté et vitaminique de la levure (ADRIAN et FRANGNE, 1970 ; CHAMPAGNAT et ADRIAN, 1974).

La présence des glucides indigestibles des enveloppes n'a pas de conséquence négative sur l'efficacité nutritionnelle, contrairement à ce qui s'observe avec l'indigestible des céréales qui entrave l'utilisation azotée et minérale de la ration. Des essais conduits sur le poulet et sur le rat démontrent que la levure débarrassée de ses enveloppes (autolysat) offre la même efficacité alimentaire que la levure entière (tableau V). À noter qu'une croissance supérieure chez les animaux consommant l'autolysat est la conséquence de son caractère appétent qui augmente les ingérés (CALET *et al.*, 1962).

Au total, si la nature chimique des glucides pariétaux de la levure indique qu'ils ne sont pas absorbables au niveau du grêle, par contre ils se révèlent dégradables en quasi-totalité par la flore intestinale. On peut les considérer comme une fibre « douce » par opposition

	Valeur de l'autolysat, en % de la levure entière
Étude sur le poulet :	
4e semaine :	
- Poids des animaux	107,5
- Indice de consommation*	99
9e semaine :	
- Poids des animaux	98
- Indice de consommation*	100
Étude sur le rat :	
- Poids des animaux	120
- Indice de consommation*	93,5
- Coefficient d'efficacité protidique**	105
* ingéré sec/gain de poids de l'animal.	
** gain de poids de l'animal/protéines ingérées.	

Tableau V : Efficacité nutritionnelle de l'autolysat d'une levure de bière après élimination des enveloppes cellulaires : en pourcentage de la valeur de la levure entière tuée (d'après CALET *et al.*, 1962).

au ballast constitué par les éléments ligno-cellulosiques des végétaux supérieurs. Ils peuvent jouer un rôle utile de ballast sans entraîner de préjudices sur le plan de la digestibilité. Cette propriété est exploitée dans un brevet qui a pour objet l'utilisation des enveloppes de levures à titre de ballast intestinal. Il s'agit d'une préparation riche en enveloppes, contenant plus de 40 % de glucides indigestibles et moins de 1,6 % d'acides nucléiques (PENTILLA et VAARA, 1992).

En pratique, la principale conséquence nutritionnelle liée à la présence des enveloppes de levure doit se situer dans le domaine de la valeur énergétique.

Le tableau VI dresse une estimation de la valeur énergétique probable d'une levure en se basant sur sa composition et sur l'utilisation digestive des composants localisés dans les enveloppes (glucides et lipides).

— Les protéines offrent une digestibilité globale comparable à celles de nombreuses productions végétales, de l'ordre de 88 % (CHAMPAGNAT et ADRIAN, 1974) ; celles des enveloppes présentent même une digestibilité supérieure (tableau IV). On peut donc leur accorder un coefficient d'ATWATER égal à celui de nombreuses céréales blutées, c'est-à-dire 3,8 kcal par g.

Constituant	Teneur (g/100 g)	Digestibilité (%)	Coefficient d'Atwater	Apport énergétique	
				kcal	kJ
Protéines	50	90	3,8	190	795
Lipides	6	35	9,0	18	75
Glucides :					
- digestibles	10	95	4,0	40	165
- indigestibles	25	?	1,5 ?	38	160
Valeur énergétique :				285	1195
Calories protid. (%)				66	

Tableau VI : Estimation de la valeur énergétique de la levure alimentaire.

— Les lipides — concentrés dans les enveloppes — ont une digestibilité de 35 %, ce qui revient à dire que 100 g de levure ne renferment en moyenne que 2 g de lipides doués d'efficacité nutritionnelle.

— En ce qui concerne les glucides, on admettra que 10 g sont digestibles et fournissent 4 kcal par g. Environ 25 g ne sont pas ou peu digestibles, mais facilement utilisables par la flore. Une fraction doit être attaquée dès l'iléon et fournit ainsi des éléments énergétiques absorbables en petites proportions. On peut accorder à ces glucides une valeur énergétique de 1,5 kcal, en raison de ce qui est observé avec d'autres glucides complexes ou atypiques (BERNIER *et al.*, 1988).

Compte tenu de ces estimations, la valeur énergétique d'une levure alimentaire se situe probablement aux environs de 285 kcal/100 g. Il s'agit donc d'un produit relativement peu énergétique, mais son intérêt protidique est tout à fait exceptionnel puisque ses calories d'origine azotée atteignent 65 % du potentiel énergétique tel que nous le calculons (tableau VI).

À ce point de vue, les levures alimentaires se classent en tête des ressources usuelles. En alimentation humaine, seuls les fromages maigres ainsi que les poissons maigres (morue) ont un caractère protidique plus prononcé ; l'œuf et le poulet ne fournissent qu'un tiers de leur énergie sous forme de protéines, le lait entier un quart seulement, etc.

En zootechnie, les levures surclassent nettement les tourteaux d'oléagineux et les protéagineux ; seules d'excellentes farines de poisson délipidées peuvent rivaliser avec les levures sèches.

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Etude de la digestibilité par les enzymes pancréatiques des levures, de leur paroi et d'une préparation de mannoprotéines, le Mannostab.

Virginie Moine-Ledoux

1. Introduction :

La levure, *Saccharomyces cerevisiae*, au cours de la fermentation alcoolique et lors de l'élevage sur lies libèrent dans le vin des mannoprotéines (Llauberes et *al.*,1988). Ces macromolécules constituant de la paroi des levures (Ballou,1976) possèdent certaines propriétés reconnues en œnologie. Elles sont responsables, en particulier de l'amélioration de la stabilité des vins, vis-à-vis des troubles protéiques (Ledoux et al., 1992) et des cristallisations des sels de l'acide tartrique (Lubbers et al., 1994 ; Dubourdieu et Moine-Ledoux, 1995). Ces résultats nous ont conduit à la mise au point d'un procédé d'extraction de mannoprotéines (Dubourdieu et Moine-Ledoux, 1994) et au développement industrielle de ce nouveau produit de stabilisation le Mannostab (*Moine-Ledoux et Dubourdieu, 2002*).

Les vins stabilisés par cette nouvelle méthode pourrait alors contenir jusqu'à 300 mg/L de Mannostab. Nous nous sommes donc intéressés à la digestibilité de cette préparation de mannoprotéines en comparaison à celle de levures entières ou de parois de levure.

Cette étude de la digestibilité des levures Saccharomyces cerevisiae, des parois de levures et du Mannostab est réalisée en utilisant le protocole adopté par le SCF n°CS/PM/2448. Ce protocole permet de mesurer l'hydrolyse des substances polymères dans des solutions simulant les fluides digestifs (salive, fluide gastrique, fluide pancréatique).

La nature des substances à digérer, essentiellement des macromolécules et les résultats décrit dans la littérature par Adrian et *al.*, 1996 démontrant la haute digestibilité des levures et des parois de levures in vivo, et signalant que ces substrats étaient préférentiellement hydrolysés par la flore intestinale nous ont conduit à éliminer l'étude de l'hydrolyse par la solution simulant la salive ou le fluide gastrique et donc à nous consacrer à l'effet d'une solution simulant le fluide intestinal.

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457 200 608 00018 RCS Bordeaux - Identification intracommunautaire FR 11 457 200 608 - S.A. au capital de 1 020 000 francs.

Afin de suivre l'hydrolyse des levures et des écorces substances insolubles, nous avons choisis de mesurer l'augmentation des substances solubilisées dans le milieu réactionnel par la mesure du degré brix. Pour le Mannostab son hydrolyse est suivi grâce à la méthode d'analyse par HPLC de tamisage moléculaire (Moine-Ledoux et *al.*, 1997). Cette analyse est également réalisée sur les essais avec les levures et les parois afin de noter si leur hydrolyse par les enzymes pancréatiques conduit à la libération de mannoprotéines. En fin un suivi des substances azotés libérés au cours de la digestion par le fluide intestinal est réalisé par dosage à la ninhydrine (Rosen, 1957).

2. Matériels et méthodes :

2.1. Protocole de digestion :

Principe :

La substance à digérer est placée dans la solution simulant le fluide digestif et maintenue à 37°C avec une continuelle agitation. Après un temps spécifique d'action compris entre 0,5 et 4 heures les produits d'hydrolyses ou les substances d'origines sont analysés afin de déterminer un pourcentage d'hydrolyse.

Produits :

- Hydrogénophosphate de potassium KH2PO4
- Soude NaOH
- Taurocholate de sodium $C_{26}H_{44}NO_6SNa$ (SIGMA)
- Extrait de pancréatine porcine 8xSUP (SIGMA)
- Levures sèches actives (Laffort œnologie)
- Ecorces de levures (Fould springer)
- Mannostab (Laffort œnologie)
- Ninhydrine (SIGMA)

Solution simulant le fluide intestinal :

Dissolution de 6,8 g de KH2PO4 dans 250 ml d'eau distillée, transfert dans une fiole de 1L, addition de 190 ml NaOH 0,2M ajout de 400 ml d'eau distillée, homogénéisation. Dissoudre 10 g d'extrait pancréatique dans 150 ml d'eau distillée, quand l'extrait est homogène l'additionner dans la fiole. Additionner 0,5 g de taurocholate de sodium homogénéiser. Le pH de la solution est ajusté à 7,5+/- 0,1 avec NaOH 0,2M, le volume final ajusté à 1L.

Tests de digestibilité des levures et des parois de levures par le suivis de la mesure du degré brix:

1 g de levures sèches actives (LSA) ou 1 g d'écorces et levures (EL) sont placées dans 50 ml de la solution simulant le fluide intestinal est placée à 37°C. Un test témoin (Témoin) est placé à 37°C contenant simplement 50 ml de la solution simulant le fluide intestinal. Les tests sont réalisées en triplicata.

Les prélèvements sont réalisés à T = 0, 1, 2, 3 heures, la prise d'échantillon est de 1 ml. L'échantillon est centrifugé afin d'éliminer les matières insolubles, la mesure du degré brix

(°brix = g/100 mL) est réalisée sur le surnageant à l'aide d'un réfractomètre à main ATAGO type N1.

Tests de digestibilité des levures, des parois de levures et du Mannostab avec mesure par HPLC de tamisage moléculaire des mannoprotéines libérées ou hydrolysées :

250 mg de levures sèches actives (LSA) ou 250 mg d'écorces et levures (EL) ou 250 mg de Mannostab (Msb) sont placées dans 50 ml de la solution simulant le fluide intestinal est placée à 37°C. Un test témoin (Témoin) est placé à 37°C contenant simplement 50 ml de la solution simulant le fluide intestinal. Les tests sont réalisées en triplicata.

Un premier prélèvement est réalisé à T = 0, la prise d'échantillon est de 2 ml. L'échantillon est placée à 100°C pendant 1 min afin de bloquer les réactions enzymatiques puis centrifugé afin d'éliminer les matières insolubles et congelé pour être analysée ultérieurement par HPLC de tamisage moléculaire.

Après 3 et 4 heures de digestibilité un deuxième et troisième prélèvements sont réalisés pour chaque modalité dans les mêmes conditions que précédemment.

Dosage des mannoprotéines par HPLC de tamisage moléculaire (Moine-ledoux et al., 1997)

Les mannoprotéines sont séparées par chromatographie liquide à haute pression de tamisage moléculaire sur deux colonnes d'acier montées en série. La première colonne (0,75 x 7,5 cm), conditionnée avec du gel trisacryl GF05 (IBF), permet de séparer les molécules par chromatographie d'exclusion. Ce gel de poids moléculaire d'exclusion 3 000 Da est habituellement utilisé pour le dessalage en chromatograhie basse pression, mais sa bonne résistance mécanique lui permet de supporter des pressions de l'ordre de 10 bars. La deuxième colonne (0,75 x 60 cm) contenant le gel TSK G2000 SW (LKB) est une colonne analytique de tamisage moléculaire. Le poids moléculaire d'exclusion de la colonne est de 70 000 Da pour les protéines globulaires. Les macromolécules sont ainsi séparées des autres constituants par chromatographie d'exclusion sur la première colonne et par chromatographie de tamisage moléculaire sur la deuxième colonne. Les conditions d'analyse sont les suivantes : volume injecté, 200 µl ; éluant, NaCl 0,1 M ; débit, 0,6 ml/h (2150 HPLC pump) ; pression 10 bars ; vitesse d'enregistrement, 0,5 mm/min (2210 recorder). Les mannoprotéines sont détectées par spectrophotométrie à 220 nm (2158 Uvicrod Sd) l'étalonnage (figure 1) et l'identification est réalisée en comparaison des temps de rétention et avec ceux des références obtenus à partir des mannoprotéines purifiées extraites selon la méthode décrites par Moine-Ledoux et al., 1997.

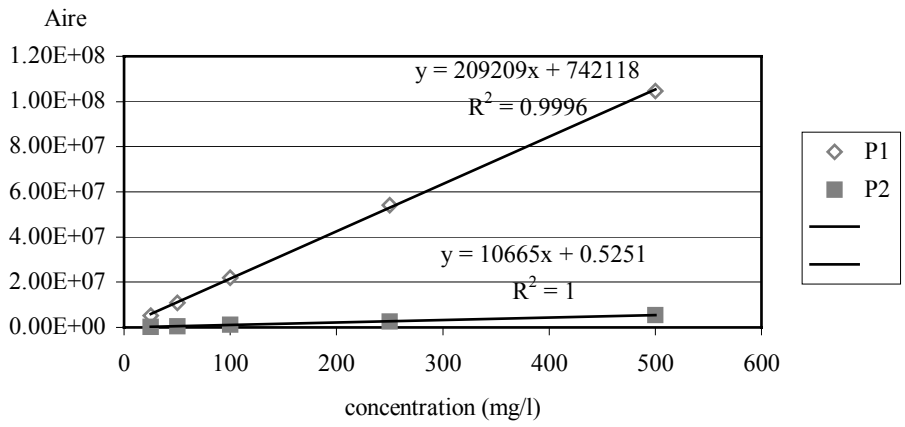

Figure 1 : Etalonnage du dosage des mannoprotéines (P1 et P2) par HPLC de tamisage moléculaire.

Test de digestibilité des levures, des parois de levures et du Mannostab avec mesure de la libération de substances azotées dosées à la ninhydrine (Rosen, 1957) :

250 mg de levures sèches actives (LSA) ou 250 mg d'écorces de levures (EL) ou 250 mg de Mannostab (Msb) sont placées dans 50 ml de la solution simulant le fluide intestinal est placée à 37°C. Un test témoin (Témoin) est placé à 37°C contenant simplement 50 ml de la solution simulant le fluide intestinal. Des Tests négatifs sont réalisés sur les LSA, EL, Msb et témoin en absence de l'extrait pancréatique. Tous les tests sont réalisés en duplicata.

Les substances azotées sont dosés à T= 0, 2 et 3 heures à l'aide du réactif à la ninhydrine.

Dosage des substances azotées à la ninhydrine :

Dans des tubes à vis sont placés 1 ml de solution à doser (l'échantillon est préalablement dilué 1/500), 0,5 ml de réactif à la ninhydrine (SIGMA). Après 10 minutes dans un bain d'eau à 100°C, les tubes refroidis sont additionnés de 2,5 ml d'éthanol absolu. Le complexe formé coloré en violet est détecté par colorimétrie à 570 nm. L'étalonnage est effectuée avec des solutions d'alanine de concentrations 6,4 µM à 250 µM (figure 2).

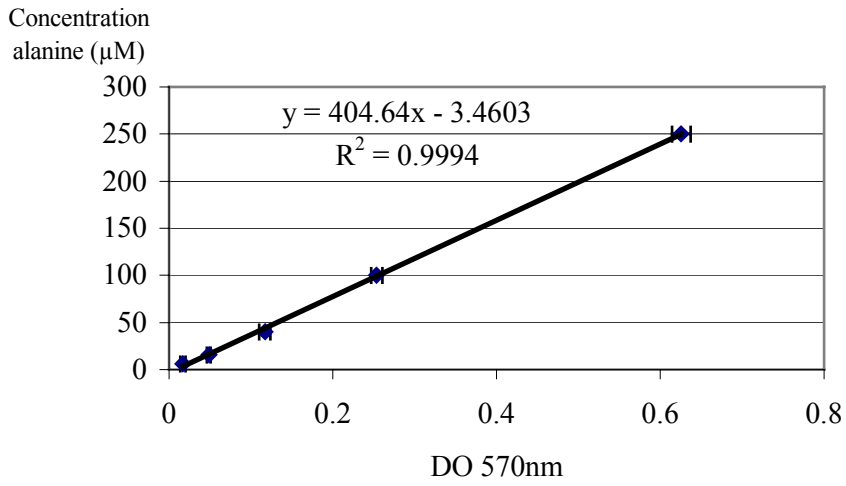

Figure 2 : Etalonnage du dosage des substances azotées par la méthode à la ninhydrine.

3. Résultats de l'hydrolyse des levures et des parois de levures et du Mannostab par la solution simulant le fluide intestinal

Les résultats des différentes analyses réalisées sont synthétisés dans les tableaux suivants. Les données individuelles sont présentées en annexe.

Le suivis de l'hydrolyse des levures et des parois de levure est tout d'abord réalisé par la mesure de l'augmentation de la matière soluble grâce au degré brix dans le milieu réactionnel, les résultats sont rapportés dans le tableau I .

Au cours de la digestion des levures entières ou des écorces de levures on note l'augmentation de la matière soluble dans le milieu réactionnel. Les enzymes pancréatiques sont donc capables d'hydrolyser ces deux substrats.

Tableau I : Evolution du degré brix au cours de l'hydrolyse des levures et des parois de levures dans la solution simulant le fluide intestinal. Les résultats sont exprimés par leur moyenne suivie de leur écart-type (n=3)

Echantillon	Temps (heures)				
	0	1	2	3	4
Témoin	4,2 ± 0.0	4,2 ± 0.0	4,2 ± 0.0	4,2 ± 0.0	4,2 ± 0.0
levures sèches actives	4,2 ± 0.0	4,4 ± 0.0	4,5 ± 0.1	4,6 ± 0.0	4,7 ± 0,1
écorces de levures	4,4 ± 0.0	4,6 ± 0.0	4,7 ± 0.1	4,8 ± 0.0	4,9 ± 0.1

La digestibilité des levures, des parois de levures et du Mannostab est également suivis par HPLC de tamisage moléculaire afin de montrer si leur hydrolyse par les enzymes pancréatiques conduit à la libération de mannoprotéines. Chacun des prélèvements à T = 0, 3 et 4 heures est analysé par HPLC de tamisage moléculaire afin de suivre l'évolution des

mannoprotéines séparées dans les pics 1 et 2 correspondant à des molécules de masse moléculaire supérieure à 70 Kda pour le pic 1 et à des molécules comprises entre 50 et 40 Kda pour le pic 2 (tableau II).

Tableau II : Résultats du dosage par HPLC de tamisage moléculaire des mannoprotéines (P1 et P2, mg/L) au cours de la digestion des levures sèches actives, des écorces de levures et du Mannostab par des sucs digestifs reconstitués.

Les résultats sont exprimés par leur moyenne suivie de leur écart-type (n=3)

échantillon	Temps (heures)					
	0		3		4	
	P1	P2	P1	P2	P1	P2
Témoin	200 ±69	0 ± 0	160 ± 69	160 ± 69	120 ± 0	160 ± 139
levures sèches actives	160 ± 69	0 ± 0	440 ± 277	320 ± 69	1840 ± 69	360 ± 0
écorces de levures	160 ± 69	0 ± 0	600 ± 208	280 ± 69	440 ± 250	200 ± 69
Mannostab	1200 ± 0	3680 ± 69	320 ± 69	760 ± 69	280 ± 69	680 ± 69

Au cours de l'hydrolyse par la solution simulant le fluide intestinal on note dans le cas des levures une importante libération de mannoprotéines, en quantité moindre pour les parois de levures et une forte diminution de celles-ci lors de l'hydrolyse du Mannostab.

Le complexe enzymatique du fluide intestinal est essentiellement constitué d'amylases et de protéases, ce sont essentiellement ces dernières qui pourraient être à l'origine de l'hydrolyse du Mannostab, des levures et des écorces. Nous avons donc choisi de suivre la libération de substances azotées au cours d'un test d'hydrolyse en présence (test +) ou en absence (test-) d'extrait pancréatique. Ces modalités permettront de montrer si l'hydrolyse des mannoprotéines, des levures ou des écorces est due aux enzymes pancréatiques ou à un simple phénomène d'autolyse.

Les résultats rassemblés au tableau III montrent une forte libération des substances azotées au cours de la digestibilité en présence d'enzymes pancréatiques (test +) et dans une moindre mesure par un phénomène d'autolyse (test -).

Tableau III : Résultats des teneurs en substances azotées (C) au cours de la digestion des levures sèches, des écorces de levures et du Mannostab par des fluides intestinaux reconstitués. (+ : en présence d'extrait pancréatique ; - :sans extrait pancréatique)

Les résultats sont exprimés par leur moyenne suivie de leur écart-type (n=3)

	Temps (heures)					
	0		2		3	
Echantillons	+	-	+	-	+	-
Témoin	27588 ±832	83 ±48	26851 ±1902	273 ±48	29561 ±285	249 ±24
levures sèches actives	31058 ±1022	1533 ±71	60751 ±3090	1937 ±48	72399 ±3994	2888 ±571
écorces de levures	33507 ±2615	772 ±71	36645 ±4517	820 ±24	53975 ±1498	1152 ±71
Mannostab	31772 ±404	392 ±24	40496 ±1854	986 ±48	60727 ±214	1818 ±71

Le rôle des protéases dans l'hydrolyse des levures, des écorces et du Mannostab par des fluides intestinaux reconstitués peut être estimé par la variation de concentration en substances azotés libérés au cours de la digestion (Tableau IV). L'interprétation des résultats obtenus montre clairement que la libération de substances azotés au cours de ces tests de digestibilité est bien due aux enzymes pancréatiques et pas seulement à un phénomène d'autolyse. Les écorces de levures sont parmi les substances testés celle qui sont le moins sensibles à l'autolyse.

Tableau IV : Interprétation des phénomènes au cours du test de digestibilité des levures séches actives, des écorces ou du Mannostab dus à l'autolyse ((test-) - (témoin-)) ou aux enzymes pancréatiques ((test+) - (témoin+) - autolyse).

Evolution des substances azotées (mg/l)	Due au phénomène d'autolyse	Due aux enzymes pancréatiques
Levures séches actives	1189	38179
Ecorces	214	18281
Mannostab	1260	25672

Les bilans de digestibilité (tableau V) calculés grâce aux dosages des substances libérées (matières solubles totales, mannoprotéines et substances azotées) montrent l'action des enzymes pancréatiques sur les écorces, les levures entières et le mannostab. L'hydrolyse des levures conduit à la libération de mannoprotéines, essentiellement de haut poids moléculaires. En revanche, les enzymes pancréatiques libèrent peu de mannoprotéines à partir des écorces. D'autre part nous montrons que le Mannostab est hautement digestible par la solution simulant le fluide intestinale à plus de 85% et en quantité relativement équivalente pour les deux fractions P1 et P2. En outre, nous démontrons que l'action des enzymes pancréatiques est essentiellement due aux activités protéasiques à l'origine de l'hydrolyse des levures, des écorces et du mannostab en substances azotées.

Tableau V : Rendements calculés selon les différentes méthodes d'évaluation de la digestibilité (mesure des substances solubilisées par le degré brix, dosage des mannoprotéines en HPLC de tamisage moléculaire, dosage des substances azotées).

(T : témoin ; Msb : Mannostab ; EL : écorce de levure ; LSA : levures sèches actives)

			échantillons		
Méthodes d'évaluations			LSA	EL	Msb
° Brix	0 heure	Poids secs (g/50ml)	1	1	-
	4 heures	Matière solubilisée (g/50 ml)	0,265	0,235	-
	Rendement%	Ms/Ps	26,5 %	23,5 %	-
Mannoprotéines	0 heure	PS : Poids sec mg/L	5000	5000	5000
		P1 mg/L	200	200	1200
		P2 mg/L	0	0	3680
		MP mg/L	200	200	4880
	4 heures	P1 mg/L	1720	320	160
		P2 mg/L	200	0	520
		MP mg/L	1920	320	680
	Rendement%	$(P1_4-P1_0)/ P1_0$	760	60	-87
		$(P2_4- P2_0)/P2_0$	100	0	-81
		$(MP_4- MP_0)/MP_0$	860	60	-86
		MP/PS	+ 34%	+ 2 %	- 84 %
Substances azotées		PS : Poids sec mg/L	5000	5000	5000
	0 heure	Test(-)	1450	689	309
		Test (+)	3470	5919	4184
	3 heures	Test(-)	2639	832	1569
		Test(+)	42838	24414	31166
	% autolysé	$Test(-)_3-Test(-)_0/PS$	23,8 %	2,9 %	25,2 %
	% hydrolysé	$(Test(+)_3-Test(+)_0)-(Test(-)_3-Test(-)_0)/PS$	763 %	366 %	513 %

4. Discussion et conclusion

Nos résultats montrent que les levures ou leur parois comme le mannostab sont hydrolysés par une solution simulant le fluide intestinal. Les travaux d'Adrian et *al,* 1996 démontraient la haute digestibilité des levures et des parois de levures in vivo, ces auteurs émettaient l'hypothèse que ces substrats étaient préférentiellement hydrolysés par la flore intestinale. En fait, nous montrons que enzymes pancréatiques et plus particulièrement les protéases sont responsables d'une partie de l'hydrolyse des levures et des écorces.

Dans le cas du Mannostab, fraction purifiée de la paroi de levure, nous montrons que ce mélange de mannoprotéines est hydrolysable par les enzymes pancréatiques à plus de 80%.

4.1.1. Références bibliographiques

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Date : 10 Juin 2003

Signature :

Annexe I : Evolution du degré brix au cours de l'hydrolyse des levures et des parois de levures dans la solution simulant le fluide intestinal.

(T : témoin ; Msb : Mannostab ; EL : écorce de levure ; LSA : levure sèche activée)

Temps (heures)	0	1	2	3	4
Modalités	° Brix	° Brix	° Brix	° Brix	° Brix
T 1	4,2	4,2	4,2	4,2	4,2
T 2	4,2	4,2	4,2	4,2	4,2
T 3	4,2	4,2	4,2	4,2	4,2
T moyenne	**4,2**	**4,2**	**4,2**	**4,2**	**4,2**
T écart type	**0**	**0**	**0**	**0**	**0**
LSA 1	4,2	4,4	4,4	4,6	4,6
LSA 2	4,2	4,4	4,4	4,6	4,8
LSA 3	4,2	4,4	4,6	4,6	4,8
LSA moyenne	**4,2**	**4,4**	**4,47**	**4,60**	**4,73**
LSA écart type	**0**	**0**	**0,12**	**0**	**0,12**
EL 1	4,4	4,6	4,6	4,8	4,8
EL 2	4,4	4,6	4,6	4,8	4,9
EL 3	4,4	4,6	4,8	4,8	4,9
EL moyenne	**4,4**	**4,6**	**4,67**	**4,8**	**4,87**
EL écart type	**0**	**0**	**0,12**	**0**	**0,06**

Annexe III : Résultats du dosage par HPLC de tamisage moléculaire des mannoprotéines (P1 et P2, mg/L) au cours de la digestion des levures sèches actives, des écorces de levures et du Mannostab par des sucs digestifs reconstitués.

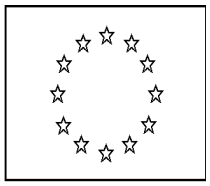
(T : témoin ; Msb : Mannostab ; EL : écorce de levure ; LSA : levure sèche activée)

Temps	0		3 heures		4 heures	
Modalités	P1	P2	P1	P2	P1	P2
T 1	240	0	120	240	120	0
T 2	120	0	120	120	120	240
T 3	240	0	240	120	120	240
T moyenne	**200**	**0**	**160**	**160**	**120**	**160**
T écart type	**69**	**0**	**69**	**69**	**0**	**139**
LSA 1	120	0	120	240	1920	360
LSA 2	240	0	600	360	1800	360
LSA 3	120	0	600	360	1800	360
LSA moyenne	**160**	**0**	**440**	**320**	**1840**	**360**
LSA écart type	**69**	**0**	**277**	**69**	**69**	**0**
EL 1	120	0	840	360	240	120
EL 2	120	0	480	240	360	240
EL 3	240	0	480	240	720	240
EL moyenne	**160**	**0**	**600**	**280**	**440**	**200**
EL écart type	**69**	**0**	**208**	**69**	**250**	**69**
Msb 1	1200	3720	360	840	360	720
Msb 2	1200	3600	240	720	240	720
Msb 3	1200	3720	360	720	240	600
Msb moyenne	**1200**	**3680**	**320**	**760**	**280**	**680**
Msb écart type	**0**	**69**	**69**	**69**	**69**	**69**

Annexe IV : Evolution des teneurs en substances azotées au cours de la digestion des levures sèches, des écorces de levures et du Mannostab par des fluides intestinaux reconstitués

(T : témoin ; Msb : Mannostab ; EL : écorce de levure ; LSA : levure sèche activée ; + : en présence d'extrait pancréatique ; - :sans 'extrait pancréatique)

	C(mg/l)			
temps (heures)	0	2	3	C_3-C_0
T-	130	320	273	
T-	35	225	225	
T- moy	**83**	**273**	**249**	**166**
T- EcT	48	48	24	
T+	28420	24949	29846	
T+	26756	28753	29276	
T+ moy	**27588**	**26851**	**29561**	**1973**
T+ EcT	832	1902	285	
M-	368	938	1747	
M-	415	1034	1889	
M- moy	**392**	**986**	**1818**	**1426**
M- EcT	24	48	71	
M+	31368	42351	60513	
M+	32176	38642	60941	
M+ moy	**31772**	**40496**	**60727**	**28955**
M+ EcT	404	1854	214	
E-	701	843	1224	
E-	843	796	1081	
E- moy	**772**	**820**	**1152**	**380**
E- EcT	71	24	71	
E+	30892	41162	55473	
E+	36122	32128	52478	
E+ moy	**33507**	**36645**	**53975**	**20468**
E+ EcT	2615	4517	1498	
L-	1461	1984	2317	
L-	1604	1889	3458	
L- moy	**1533**	**1937**	**2888**	**1355**
L- EcT	71	48	571	
L+	30036	57660	68405	
L+	32081	63841	76393	
L+ moy	**31058**	**60751**	**72399**	**41341**
L+ EcT	1022	3090	3994	



EUROPEAN COMMUNITIES
DIRECTORATE-GENERAL III
INDUSTRY
Industrial affairs III: Consumer goods industries
Foodstuffs - Legislation and scientific and technical aspects

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FOOD CONTACT MATERIALS

NOTE FOR GUIDANCE

("NOTE FOR GUIDANCE OF PETITIONER WHEN PRESENTING AN APPLICATION FOR ASSESSMENT OF A SUBSTANCE TO BE USED IN FOOD CONTACT MATERIALS PRIOR TO ITS AUTHORISATION")

(Updated to 23 February 1999)

This document is available now only in English.

Annex 1

MEASUREMENT OF HYDROLYSIS OF PLASTICS MONOMERS AND ADDITIVES IN DIGESTIVE FLUID SIMULANTS

NOTA BENE. This annex has been already adopted by the SCF as CS/PM/2448 Final.

Contents

INTRODUCTION

For the protection of human health, plastic food contact materials shall be in compliance with Directive 90/128/EEC updated with regard to composition and migration of constituents to foodstuffs coming into contact with these materials.

Constituents that may migrate to foodstuffs comprise residual monomers and other starting substances, residual process chemicals and additives as well as breakdown products and impurities of these substances.

Certain constituents may hydrolyse when ingested. The method described in this Guideline allows determination of the extent of hydrolysis, especially of esters, in order to assess whether the constituents break down into innocuous substances.

1 SCOPE

The method can be used to measure the extent of hydrolysis of monomers and additives in vitro , using standard digestive fluid simulants for saliva, gastric juice and intestinal fluid.

The method does not describe the analytical procedures required for the determination of the parent constituent and its hydrolysis products in the simulants.

2 PRINCIPLE

The test substance (monomer or additive) is dissolved in an appropriate solvent. An aliquot of the solution is transferred to the digestive fluid simulant, which is maintained at 37°C with continual agitation. After a specified time period the concentrations of both parent constituent and hydrolysis products are determined in the simulant, whereupon percentage hydrolysis is calculated.

3 REAGENTS

NOTE: All reagents should be of recognised analytical quality unless otherwise specified.

3.1 Chemicals

3.1.1 Water, distilled or deionised
3.1.2 Sodium bicarbonate ($NaHCO_3$)
3.1.3 Sodium chloride (NaCl)
3.1.4 Sodium taurocholate?
3.1.5 Potassium carbonate (K_2CO_3)
3.1.6 Sodium hydroxide standard solution, 0.2 M
3.1.7 Hydrochloric acid standard solutions, 2 M and 0.1 M
3.1.8 Potassium dihydrogen orthophosphate (KH_2PO_4)
3.1.9 Porcine pancreatin extract, activity equivalent to 8x SUP specification
3.1.10 Dispersing solvents, one of:

- acetonitrile
- N,N-dimethylacetamide
- 1,4-dioxane
- ethanol
- methanol
- propan-2-ol
- tetrahydrofuran
- water

3.2 Digestive fluid simulants

3.2.1 Saliva simulant:
Dissolve 4.2 g of sodium bicarbonate ($NaHCO_3$), 0.5 g of sodium chloride (NaCl) and 0.2 g of potassium carbonate (K_2C0_3) in 1 litre of water. The pH of the solution should be approximately9.

3.2.2 Gastric-juice simulant:
Dilute 0.1 M hydrochloric acid standard solution to a concentration of 007 M. The pH of the solution should be 1.2 ± 0.1.

3.2.3 Intestinal-fluid simulant:

NOTE: Care should be taken to ensure that the simulant is prepared in the order given.

Dissolve 6.8 g of potassium dihydrogen orthophosphate (KH_2PO_4) in 250 ml of water, transfer to a 1 L volumetric flask and add 190 ml of 0.2 M sodium hydroxide (NaOH). Add 400 ml of water and shake briefly to mix. Weigh 10.0 g of pancreatin extract into a 250 ml beaker. Add a little water, and stir to make a stiff, homogenous paste. Gradually dilute the paste with small portions of water, stirring well after each dilution, to give approximately 150 ml of a lump-free solution. Transfer the solution to the volumetric flask, rinsing the beaker and funnel with water. Add 0.5 g of sodium taurocholate, gently shake the flask and make the volume up to the neck of the flask. Adjust the pH of the solution to 7.5 $\pm$ 0.1 with 0.2 M sodium hydroxide (NaOH). Make the volume up to the mark with water and shake thoroughly to mix.

4 APPARATUS

NOTE: An instrument or item of apparatus is listed only where it is special, or made to a particular specification, usual laboratory equipment being assumed to be available.

4.1 Glass vials, 100 ml or 125 ml, with crimp-on typePTFE/silicone rubber septa.

4.2 Crimping and decapping pliers.

4.3 Device for mechanical agitation of the simulant, e.g. a flask shaker, or a magnetic stirrer bar for use with a stirrer plate, situated in a cabinet or water bath controlled to a temperature of 37 $\pm$ 1°C.

5 SAMPLES

NOTE: The test substance should be of similar purity as the substance used in food contact materials.

5.1 Preparation of stock solutions

Weigh out the required weight of the test substance to the nearest 0.1 mg into a 10 ml volumetric flask and dissolve in a suitable dispersing solvent such as one listed in section 3.1.10. Make the volume up to the mark, and shake the flask thoroughly to mix.

NOTE: The solvent selected must completely dissolve the test substance and must not chemically react with it.

The final concentration of solvent (other than water) in the digestive fluid simulant should not exceed 0.1% (v/v).

The concentration of the test substance in the digestive fluid simulant should be selected such as to enable determination of the substance down to 5% of the amount added to the simulant. Anyhow, that concentration should not be lower than the maximum likely human intake predicted from migration studies.

6 PROCEDURE

6.1 Hydrolysis equation

Set out the hydrolysis equation, using the following model expression:
PC => HP-1 + HP-2 (+ HP-3 +..... HP-N), in which:
PC = parent constituent
HP = hydrolysis product

6.2 Selection of simulants

Select simulants to be used in the test such that the analytical effort is kept to the minimum, e.g. a test with intestinal fluid simulant is often sufficient to demonstrate hydrolysis of esters. So, if the test substance is an ester, a test with intestinal fluid simulant should be carried out first. If complete hydrolysis is demonstrated, it is not necessary to perform tests with other simulants.

6.3 Performance of hydrolysis test

Transfer for each test 100 ml of the digestive fluid simulant to a glass vial using a measuring cylinder. Crimp-seal the vial with a PTFE-silicone rubber septum. Commence shaking the vial or stirring its contents and equilibrate the simulant at 37 ± 1°C.

NOTE: As for analytico-technical reasons each substance in the hydrolysis equation selected for determination has to be assessed in a separate hydrolysis test and each of the determinations has to be carried out in triplicate, the number of glass vials needed for the test amounts to thrice the number of combinations of substances (be it parent constituent or hydrolysis product) to be determined, specified time period and simulant.

Subsequently add a suitable aliquot of the stock solution (25 to 100 µl) to the simulant, using a 100 µl syringe. Inject the solution through the septum, below the surface of the simulant, and continue agitation or stirring for the duration of the test. Take the duration of the test from the following table:

- saliva simulant 0.5 h
- gastric-juice simulant 1, 2 and 4 h
- intestinal-fluid simulant 1, 2 and 4 h

NOTE: If gastric-juice simulant or intestinal-fluid simulant is used for the test, a test for one hour should be performed first. If complete hydrolysis is demonstrated, it is not necessary to perform tests for two and four hours.

6.4 Analysis of hydrolysates

After termination of the hydrolysis test determine the hydrolysis products in the hydrolysate. Use an appropriate analytical method and calculate percentage hydrolysis from the results.

NOTE It is insufficient to only measure disappearance of the parent constituent. A case by case selection should be made about which hydrolysis products need be measured in order to permit a judgement about mass balance.

Suitability of the analytical methods should be demonstrated by performing tests with standard addition of the hydrolysis product(s) of interest set out in the CEN standard format, which can be found in the document "Commission Explanatory Guidance for Migration Testing") (see later).

7 TEST REPORT

The test report should conform to the CEN standard format set out in the document "Commission Explanatory Guidance for Migration Testing")

.....
.....
.....
.....
.....
.....
.....

(1) Indicate first the most common chemical name of the substance or, in the case, of a substance included in the Directive 90/128/EEC the name given in this Directive.

(2) Specify whether it is monomer or additive

(3) REF.N. = Substance Reference Number. Indicate this number if it has been given to the substance under examination.

1.	**IDENTITY OF SUBSTANCE**	
1.1	**individual substance:**	
1.1.1	**chemical name:**	
1.1.2	**synonym(s):**	
1.1.3	**trade name(s):**	
1.1.4	**CAS Nr:**	
1.1.5	**molecular and structural formula:**	
1.1.6	**molecular weight:**	
1.1.7	**spectroscopic data:**	
1.1.8	**purity (%):**	
1.1.9	**major impurities (%):**	
1.1.10	**specifications:**	
1.1.11	**other information**	
1.2	**defined mixture:**	
1.2.1	**chemical name:**	
1.2.2	**synonym(s):**	
1.2.3	**trade name(s):**	
1.2.4	**CAS N°:**	
1.2.5	**constituents:**	
1.2.6	**proportions in the mixture:**	
1.2.7	**molecular and structural formula:**	
1.2.8	**molecular weight (Mw) and range:**	
1.2.9	**spectroscopic data:**	
1.2.10	**purity (%):**	
1.2.11	**major impurities (%):**	
1.2.12	**specifications**	
1.2.13	**other information:**	
1.3	**Non-defined mixture:**	
1.3.1	**chemical name:**	
1.3.2	**synonym(s):**	
1.3.3	**trade name(s):**	
1.3.4	**CAS N°:**	
1.3.5	**starting substances:**	
1.3.6	**manufacturing details:**	
1.3.7	**substances formed:**	
1.3.8	**purification by:**	
1.3.9	**by-products:**	
1.3.10	**molecular and structural formula:**	
1.3.11	**molecular weight (M_W) and range:**	
1.3.12	**purity (%):**	

1.3.13	**major impurities (%):**	
1.3.14	**spectroscopic data:**	
1.3.15	**specifications**	.
1.3.16	**other information:**	
1.4	**polymer used as additive:**	
1.4.1	**chemical name:**	
1.4.2	**synonyms:**	
1.4.3	**trade name(s):**	
1.4.4	**CAS N°:**	
1.4.5	**starting substances:**	
1.4.6	**additive(s):**	
1.4.7	**structure of polymer:**	
1.4.8	**weight averaged molecular mass:**	
1.4.9	**number averaged molecular mass**	
1.4.10	**molecular mass range:**	
1.4.11	**constituents with molecular mass <1000 (%):**	
1.4.12	**manufacturing details:**	
1.4.13	**viscosity, if available:**	
1.4.14	**melt flow index, if available:**	
1.4.15	**density (g/cm^3):**	
1.4.16	**spectroscopic data:**	
1.4.17	**residual monomers (mg/kg):**	
1.4.18	**purity (%):**	
1.4.19	**major impurities (%):**	
1.4.20	**specifications**	
1.4.21	**other information:**	
2.	**PHYSICAL AND CHEMICAL PROPERTIES OF SUBSTANCE**	
2.1	**physical properties**	
2.1.1	**melting point (°C):**	
2.1.2	**boiling point (°C):**	
2.1.3	**decomposition temperature (°C):**	
2.1.4	**solubility (g/l):**	
2.1.5	**other information:**	
2.2	**chemical properties**	
2.2.1	**nature:**	
2.2.2	**reactivity:**	
2.2.3	**stability:**	

2.2.4	**hydrolysis:**	
2.2.5	**decomposition/ transformation:**	
2.2.6	**decomposition/ transformation product(s):**	
2.2.7	**interaction with food substances:**	
2.2.8	**octanol/water partition (log $P_{o/w}$):**	
2.2.9	**other information:**	
3.	**INTENDED APPLICATION OF SUBSTANCE**	
3.1	**food contact material:**	
3.2	**technological function:**	
3.3	**maximum process temperature (°C):**	
3.4	**maximum percentage in formulation:**	
3.5	**conditions of contact in practice**	
3.5.1	**contact food:**	
3.5.2	**time and temperature:**	
3.5.3	**surface to volume ratio:**	
3.5.4	**other information:**	
3.6	**treatment of food contact material prior to use:**	
3.7	**other information:**	
4.	**AUTHORISATION OF SUBSTANCE**	
4.1	**EU countries**	
4.1.1	**in Member States:**	
4.1.2	**notified as “new substance” in the context of 6th Amendment of Directive 67/548/EEC**	
4.1.3	**other information:**	
4.2	**non-EU countries**	
4.2.1	**in USA:**	
4.2.2	**in Japan:**	
4.2.3	**in other countries:**	
4.2.4	**other information:**	
4.3	**other information:**	
5.	**DATA ON MIGRATION OF SUBSTANCE**	
5.1	**specific migration (SM)**	
5.1.1	**substance:**	
5.1.2	**test sample:**	

5.1.2.1 chemical composition:	
5.1.2.2 physical composition:	
5.1.2.3 density, meltflow index of polymer:	
5.1.2.4 dimensions of test sample:	
5.1.2.5 dimensions of test specimen:	
5.1.3 treatment of test sample prior to testing:	
5.1.4 test food(s)/food simulant(s):	
5.1.5 contact mode:	
5.1.6 contact time and temperature:	
5.1.7 surface to volume ratio:	
5.1.8 analytical method:	
5.1.9 detection/ determination limit:	
5.1.10 precision of test method:	
5.1.11 recovery:	
5.1.12 other information:	
5.1.13 results:	Give the results in a table appropriate to the specific case. An example of table recommended is indicated below.

Table

Simulants	Time	Temperature (° C)	Results mg/dm^2	Results* (mg/kg of food)

* *Specify clearly the calculations made, mainly as regards the ratio S/V used.*

5.2 overall migration (OM)	
5.2.1 test sample:	
5.2.2 treatment of sample prior to testing:	
5.2.3 food simulant(s):	
5.2.4 contact mode:	
5.2.5 contact time and temperature:	
5.2.6 surface to volume ratio:	
5.2.7 test method	
5.2.8 other information:	
5.2.9 results:	Give the results in a table appropriate to the specific case. An example of table recommended is indicated below.

Table

Simulants	Time	Temperature (° C)	Results mg/dm^2	Results* (mg/kg of food)

**) Specify clearly the calculations made, mainly as reg ards the ratio S/V used.*

6. DATA ON RESIDUAL CONTENT OF SUBSTANCE IN THE FOOD CONTACT MATERIAL	
6.1 actual content:	
6.2 substance	
6.3 test sample	
6.3.1 chemical composition:	
6.3.2 pysical composition:	
6.3.3 density, melt flow index of polymer:	
6.3.4 dimensions of test sample:	
6.3.5 dimensions of test specimen:	
6.4 treatment of sample:	
6.5 test method:	
6.5.1 detection/ determination limit:	
6.5.2 precision of test method:	
6.5.3 recovery	
6.5.4 other information:	
6.6 results:	
6.7 calculated migration (worst case):	
6.8 residual content versus specific migration:	

7. TOXICOLOGICAL DATA

A summary should be completed for each study reported in this section. The main findings should be summarised and a statement made on whether significant deviations from control and normal values occurred.

7.1 Genotoxicity	
7.1.1 Gene mutation in bacteria:	
7.1.2 In vitro mammalian chromosome aberration test:	
7.1.3 In vitro mammalian cell gene mutation test:	

7.1.4 Other information:	
7.2 General toxicity	
7.2.1 Subchronic (90d) oral toxicity:	
7.2.2 Chronic toxicity/carcinogenicity:	
7.2.3 Reproduction/teratogenicity:	
7.2.4 Other information:	
7.3 Metabolism	
7.3.1 Absorption, distribution, biotransformation and excretion:	
7.3.2 Accumulation in man:	
7.3.3 Other information:	
7.4 Miscellaneous	
7.4.1 Effects on immune system:	
7.4.2 Neurotoxicity:	
7.4.3 Induction on peroxisome proliferation:	
7.4.4 Other information:	
8. REFERENCES	

CHAPTER IV

III/5442/96 Rev. 1-EN

COMMISSION EXPLANATORY GUIDANCE

("**Commission Explanatory Guidance for Migration Testing**")

NOTA BENE
This annex, set out under EC responsibility, was prepared by a task force composed of some government, industrial, CEN and SCF experts. It should be not considered an SCF or SCF-WG "FCM" document. This document is strictly related to the Directives 97/48/EC (briefly called "2nd Amendment to the Directive 82/711/EEC", which established the basic rules for migration testing).

1. Introduction

1.1 This document provides an explanation and guidance on the conducting of prescribed "migration tests" as well as the "substitute" and "alternative" tests. It is particularly aimed at the analysts who carry out testing to ensure compliance, e.g. enforcement authorities, industry, retailers and certification laboratories. It should also be used by the analysts preparing a technical dossier to be submitted to the Commission. In principle, there is no relevant basic difference between the tests to be carried out to determine compliance with the EC Directives and the tests required by the SCF to evaluate a substance to be authorised.

1.2 The Commission intends to periodically update this document to take account of developments in migration testing. The Commission recommends that these guidelines are strictly observed. It should be remembered that:

a) the EC Directives define the legal rules applicable at European level;

b) other EC documents, for example this document or the "SCF Guidelines", explain these rules and their application in practice;

c) the document "Methods of analysis" gives the references and/or the analytical procedures to determine overall migration as well the migration of the specific substances or groups of substances;

If there are discrepancies between CEN and EC documents, brought about for example by periodic updating at different times, then for compliance purposes

the EC documents have precedence, unless a Commission document clearly states to the contrary.

2. Migration Testing

2.1 Migration into foodstuffs and into food simulants

Directive 82/711/EEC, as amendment by Directive 93/8/EEC and by Directive 97/48/EC provides for migration limits:

"Verification of compliance of migration into foodstuffs with the migration limits shall be carried out under the most extreme conditions of time and temperature foreseeable in actual use. Verification of compliance of migration into food simulants with the migration limits shall be carried out using conventional migration tests ....."

Therefore these Directives provide two options:

First option: to carry out the migration tests with foodstuffs themselves.

Second option: to carry out the migration tests using food simulants.

It is always possible to determine the migration, mainly the specific migration, directly in foodstuffs in the worst test conditions in order to a) ascertain compliance with the legislation or b) to "provide information sufficient to permit estimation of the maximum daily intake of the substance and its impurities as well as its breakdown and reaction products" as prescribed by the (future) "SCF Guidelines".

Alternatively, it is possible to determine the level of migration using the food simulants and the test conditions set out in the Directive 97/48/EC.

2.2 Food simulants

Directives 97/48/EC and 85/572/EEC provide the following four simulants[8]:

- distilled water or water of equivalent quality (simulant A).
- 3% acetic acid (w/v) in aqueous solution (simulant B).
- 10% ethanol (v/v) in aqueous solution (simulant C).
- rectified olive oil (simulant D).

However, olive oil can be replaced by other equivalent non-volatile fatty food simulants (see point 2.3). These alternative fatty simulants are also indicated by the

[8] The specifications of these food simulants can be found in the document "Methods of Analysis".

abbreviation "Simulant D". Therefore the abbreviation "simulant D" in Directive 97/48/EC refers not only to olive oil but also to each of the other equivalent non-volatile fatty food simulants (e.g. sunflower oil, synthetic mixture of triglycerides).

As simulant D is generally more aggressive than any solid or semi-solid fatty foods, and because of all the food simulants it is usually the most severe, reduction factors are introduced beside certain fatty foods to take into account this greater extraction power (see Directive 85/572/EEC). Therefore in order to establish in these cases whether the sample complies with the limits, the value obtained in the overall or specific migration tests shall be divided by the corresponding reduction factor in Directive 85/572/EEC to the fatty food under examination. If the material or article is intended to come into contact with more than one foodstuff or group(s) or foodstuffs having different reduction factors, various reduction factors shall be applied. If one or more results of such calculation, after consideration of the analytical tolerance, exceed the restriction, then the material is not suitable for that group(s) of foodstuff.

2.3 **Other equivalent non-volatile fatty food simulants (simulants D)**

Directive 97/48/EC, Chapter 1 states the following for olive oil (simulant D):

"However this reference simulant D may be replaced by a synthetic mixture of triglycerides or sunflower oil or corn oil with standardised specifications ("Other fatty food simulants", called "simulants D"). If, when using any of these other fatty food simulants, the migration limits are exceeded, for the judgement of non compliance a confirmation of the result by using olive oil is obligatory, when technically feasible. If this confirmation is not technically feasible and the material or article exceeds the migration limits it shall be deemed not in compliance with the Directive 90/128/EEC."

As clearly stated in the Directive, the use of other equivalent non-volatile fatty food simulants is authorised without any need to check "a priori" its equivalency or its greatest extraction power. In fact the available experimental data showed that the level of migration in these simulants is approximately of the same order or slightly greater than the level obtained with olive oil. Only in the case of legal prosecution due to overall or specific limits being exceeded is it recommended that the results are confirmed by testing with olive oil, provided this is technically feasible.

If the measurement is technically not feasible with olive oil (for valid reasons which should be documented), the values obtained with the alternative equivalent non-volatile fatty food simulant shall be taken as the correct value. A typical example of a valid reason for the use of other equivalent non-volatile fatty food simulants is the presence of an unacceptable amount of interfering components in the olive oil or in the test material. Otherwise the result with the alternative equivalent non volatile fatty simulant remains the only valid result.

2.4 **Contact (t,T) conditions for migration tests**

Directive 97/48/EC in addition to Annex 1 of Directive 90/128/EEC provides the test conditions (food simulants, contact times and temperatures, etc.) to be followed when conducting migration tests with food simulants. Details relating to the performance of migration experiments, the analytical procedures (apparatus, reagents, samples etc.) and the test methods to determine overall and specific migration levels into food simulants can be found in the CEN documents (see document "Methods of analysis"). As regards the recommendation of the Commission see the specific Section of "Practical Guide".

If there are any discrepancies between the CEN and EC documents (Directives or other publications) follow the recommendation of point 1.3 of the introduction of this document.

Other indications in the selection of the contact times and temperatures to be used in migration tests using the food simulants can be found, if considered appropriate, in the document "Explanatory note on the use of t-T Table in Directive 82/711/EEC as amended for selection of conditions in migration testing" included as annex in the document "Commission Explanatory Guidance on Migration Testing".

It should be recognised that all these references are sometimes insufficient to give guidance in the choice of test conditions in real cases. Therefore the analyst should always carefully consider the potential uses of the material or article under examination and select from the times and temperatures specified in the Directive 97/48/EC and in the above mentioned references documents those which correspond to the worst foreseeable conditions of contact.

Some examples below illustrate the selection of test conditions from the time and temperature conditions occurring in real situations:

- A food contact material designed to be in contact with food during a sterilisation period of 20 minutes at 121ºC, followed by storage at room temperature for 6 months, shall be submitted to the test conditions of 30 minutes at 121ºC followed by 10 days at 10ºC.

- A food contact material that comes into contact with food for 9 seconds at 90ºC, and subsequent storage for 14 days in a refrigerator at temperatures of approximately 10ºC, shall be tested for 10 days at 20ºC. The period at 90ºC is much too short to be relevant and is therefore ignored. In fact in the case given, the rule following which the migration varies linearly with the square root of time and doubles with each 10ºC increase of test temperature can be applied, provided no change in the morphology of the plastic occurs. In so doing, the 9 seconds at 90ºC is equivalent to only about 20 minutes at 40ºC which is negligible compared to 10 days.

- The packaging of a frozen ready meal which according to the instructions can be heated in the packaging in a conventional oven at 200ºC for 30 to 40 minutes shall be tested for 1 hour at 175ºC only. In this case the storage period before heating is not relevant as the test conditions of 1 hour at 175ºC are

considered much more severe than the conditions (10 days at 5ºC) to simulate the storage period.

- Articles intended to be filled with hot food at a starting temperature of 85ºC and where the temperature decreases within 15 minutes to a temperature below 70ºC can be tested for 2 hours at 70ºC, if they are not intended to be used for storage, such as coffee cups. It is however also permitted to apply test conditions of 30 minutes at 100ºC as a more severe test.

- In case of hot fill, the temperature of the food after 15 minutes is still above 70ºC, the article shall be tested for 30 minutes at 100ºC.

- If the food in the above sample is subsequently stored for along period at room temperature, then the material shall be submitted by a combination of test conditions of 30 minutes at 100ºC followed by 10 days at 40ºC.

2.5 Test conditions considered "more severe conditions"

Directive 97/48/EC provides at point 4 of the Annex the following general clause, applicable to all Chapters:

".... it is permissible:

a) to reduce the number of tests to be carried out to that or those which, in the specific case under examination, is (are) generally recognised to be the most severe on the basis of scientific evidence:

b) to omit the migration or the substitute or the alternative tests where there is conclusive proof that the migration limits cannot be exceeded in any foreseeable conditions of use of the material or article".

Further explanation is given in the subsequent paragraphs. In order to recognise the test conditions which should be considered “more severe conditions” the two main elements of these conditions are considered separately i.e. the simulants from one side and the time and temperature from the other side.

2.5.1 Simulants considered "more severe" or "less severe"

Directive 97/48/EC gives in Chapter 1 some examples of simulants considered more severe than others. For materials and articles intended for general purpose use with all four food types, the test with the water simulant is not necessary because water is considered less severe than 3% acetic acid or 10% ethanol simulants. Similarly, for materials and articles intended only for acidic and alcoholic foods the test with 3% acetic acid can be omitted because it is, in principle, considered less severe than the test with 10% ethanol.

It is generally recognised that ethanol 10% can be considered conventionally more severe than the test with water. Moreover, it is generally recognised that the test with acetic acid 3% can be omitted because it is considered less severe than the test

with ethanol 10% if the ample does not contain organic and inorganic metals compounds, amines and other substances soluble in acetic acid.

Analysts can find other situations where it is evident that for specific plastics under examination, the test may be omitted, because it is "less severe" than another. A common example of this is the migration of a non-polar substance from a non-polar plastic which is almost invariably higher into simulant D (olive oil and other fat simulants) than into the aqueous simulants A, B or C. If this is so then the three aqueous simulants may be omitted from testing for the migration of this substance. The only condition to be satisfied is that this omission can be justified "on the basis of scientific evidence".

2.5.2 **Test conditions (times and temperatures) considered "more severe"**

Directive 97/48/EC Chapter 2, point 2 already gives some examples of more severe test conditions. It is recognised in point 2.1 that for plastic materials and articles intended to come into contact with foodstuffs at any condition of time and temperature, the tests with simulants B and C for 4 hours at 100ºC or at reflux temperature and with simulant D for 2 hours at 175ºC should be considered more severe than any other to be selected in practice.

Other situations can easily occur in practice. For instance:

- to carry out a test at a higher temperature avoids testing the sample at a lower temperature if the contact time remains unchanged.

- A food contact material intended to be used in separate applications of contact time and temperature.

  1. long term storage at -20ºC.

  2. long term storage at room temperature.

  3. heating of food in boiling water ("au bain marie").

  should be respectively tested for:

  1. 10 days at 5ºC.

  2. 10 days at 40ºC.

  3. 2 hours at 100ºC or reflux temperature.

  to cover the individual contact conditions. However, the three individual tests may be replaced by one combined test of 2 hours at 100ºC followed by 10 days at 40ºC. That test will cover the three applications mentioned. Alternatively the tests for 10 days at 40ºC and the 2 hour at 100ºC can be performed separately. In that way also the condition of 10 days at 5ºC will be covered.

2.6 Volatile migrants

Directive 97/48/EC, Chapter 2, point 3, provides the following clause:

"When testing for the specific migration of volatile substances, the test(s) with simulant(s) shall be performed in a manner which recognises the loss of volatile migrants which may occur in the worst foreseeable conditions of use".

Testing in closed systems (i.e. by total immersion in gas-tight cells) gives more reproducible results and this method should preferably be used in the first instance. This represents a worst-case, however, as for most applications such as bottles, pouches, containers etc., loss of the volatile substance will occur in actual use with the intended foodstuff. If results obtained in a closed system are within the specific migration limit then the plastic is acceptable for the application being considered. If the migration is above the limit then the plastic should not be rejected but should be re-tested using an exposure protocol more representative of actual use. It should be noted that if there still remains doubt about the validity of the exposure protocol, then for many volatile substances there are methods available to measure migration into the actual foodstuff itself.

2.7 Special cases

2.7.1 Directive 97/48/EC, Chapter 2, point 4.2, provides the following clause:

"If it is found that carrying out the tests under the contact conditions specified in table 3 causes physical or other changes in the test specimen which do not occur under worst foreseeable conditions of use of the material or article under examination, the migration tests shall be carried out under the worst foreseeable conditions of use in which these physical or other changes do not take place".

In some cases it is seen that a food contact material can be used under certain conditions of time and temperature in contact with specified foodstuffs, while the material cannot sustain the test conditions that should be applied in the migration experiments. In this respect the changes are mainly caused by the selection of "more severe" temperature conditions.

For example: take-away meals, such as fried rice with free fat on the surface, which are hot filled in a polystyrene or LDPE tray, will remain for more than 15 minutes at a temperature above 70ºC. In daily practice the tray will be capable of holding the food without any visible changes. As a consequence of the real contact conditions the tray has to be tested with olive oil for a period of 1 hour at 100ºC. It may appear that the tray deforms, or, even worse, deteriorates during contact with fat simulant at 100ºC. In such cases the selected test conditions may be adapted by taking a longer contact period at a lower temperature. As alternative conditions 2 hour 70ºC may be considered in this example. But also conditions of appropriate temperature which prevent the tray from deforming or melting with an adapted time may be selected. In those cases any written report should give notice of the deviations as well as arguments for that deviation.

Swelling of food contact materials during contact with food simulants is not considered to be a relevant change. Usually it will hardly be visible, most likely it will happen in daily use also. Even when using volatile fatty food simulants, swelling should not be considered a significant change of the food contact material.

2.7.2 Directive 97/48/EC, Chapter 2, point 4.4, provides the following clause:

"In those instances where the conventional conditions for migration testing are not adequately covered by the test contact conditions of the table 3 (for instance contact temperatures greater than 175º or contact time less than 5 minutes), other contact conditions may be used which are more appropriate to the case under examination, provided that the selected conditions may represent the worst foreseeable conditions of contact for the plastic materials or articles being studied".

Also in this case the general guideline which could be suggested is that the petitioner or the person responsible of the control give notice of the special conditions used and the reasons for their choice.

2.7.3 Another method for the determination of specific migration level

In principle Directive 90/128/EEC stipulates in annex 1, point 4 that the specific migration level should be determined by "analytical determination of ....the specific quantity of one or more substances.. released by the sample..... (to) the foodstuffs or simulant". It is normal practice to measure the concentration of the substance directly into the food simulant. It is possible in some cases, however, to measure the quantity of substance released by the difference in the concentration in the material or article before (QI) and after (QF) the migration test. This difference (DQIF, difference in quantity, initial minus final) when coupled with the mass of plastic and simulant employed, can be used to calculate the concentration of substance released into the simulant. To conduct this procedure the analyst must ensure that the method used to determine QI and QF has accuracy and precision characteristics sufficient to estimate the value DQIF reliably.

2.8 Substitute tests

2.8.1 Directive 97/48/EC, in point 2 of the Annex, provides the following clause:

"Substitute tests" which use the "test media" under the "conventional substitute test conditions" as set out in Chapter 3 shall be carried out if the migration test using the fatty food simulants (see Chapter 1) is not feasible for technical reasons connected with the method of analysis.

Various situations may occur which justify the use of substitute tests. However there are two main accepted reasons where the substitute tests should be applied, as set out in Chapter 3:

a) when the test with each of the possible simulants D is inapplicable for technical reasons connected with the test (e.g. interferences, incomplete extraction of oil,

absence of stability of the weight of the plastic, excessive absorption of fat simulant, reaction of the component with the fat);

b) when the sensitivity of the analytical method in olive oil for specific substances is insufficient and obliges the petitioner to present additional toxicological data to that what reasonably could be expected to be requested by the SCF (e.g. migration not detectable with a detection limit greater than 0.05 mg/kg).

As the criteria under a) is too generic and because the use of substitute tests should be limited as much as possible, particularly in petitions, the analyst should give in the technical dossier the experimental or the theoretical elements which justify the departure from using simulants D. In general the elements requested are:

a) explanation for failure;

b) an outline of the experiments carried out;

c) some relevant data and visual proof, e.g. chromatograms;

d) additional data on the approximate solubility of the substance in the fat simulant as well as in the extraction media used in the substitute test and the stability or expected stability of the substance in olive oil should be provided to help the decision on accepting the extraction medium.

It should be stressed that if the technical difficulties in using olive oil (e.g. interference in the peak of oleic acid) can be avoided by using another simulant D (e.g. HB 307), this latter one should be used.

It should also be stressed that the substitute tests are conventionally deemed equivalent to the tests using simulants D, the reduction factors also apply to the extraction medium used in the substitute tests.

Below some typical examples of replacement or no replacement of simulant D are given.

- Expanded polystyrene samples with an open cell structure will usually absorb a large amount of the fat simulant. The analytical tolerance of 3 mg/dm^2 will be exceeded when more than approximately 400 mg of fat simulant is absorbed by 1 dm^2 of test material, taking into account a 1 to 4% analytical error in the determination of the amount of fat simulant. In such cases the determination of overall migration into the fat simulant is not possible unless a more accurate measurement of the amount of fat absorbed by the test sample is available.

- Moisture sensitive materials have to be conditioned to constant weight before and after contact with the fat simulant. It may appear that e.g. for thick polyamide samples constant weight can not be achieved by the prescribed methods given in relevant CEN methods. In such cases the fat test is not applicable and a substitute test should be performed.

- In the gas chromatogram of oil extracted from a test sample an interference may be observed at the retention time of the prescribed internal standard. If this occurs an alternative internal standard should be used and therefore conducting of substitute tests is not acceptable.

- A polymer sample containing per dm^2 more than 2 mg of additive - which interferes in the GC determination at the retention time of oleic acid - should be tested using a fat simulant in which the oleic acid is not present. Fat simulants like HB 307 or Miglyol 812 are most appropriate in these circumstances. Substitute test may not be acceptable.

In the determination of the specific migration other difficulties may require the application of substitute tests, such as:

- Reaction of the migrant with the fat simulant. Amines such as hexamethylenediamine and ethylenediamine are known to react with oil constituents of the test material during the contact period with the oil. As a result migration can not be determined. Performance of substitute tests is required.

- Insufficient sensitivity of the analytical method. Typically with low specific migration limits, it may appear that no analytical method is available or can be developed to demonstrate migration to be less than the SML, even when taking a more favourable ratio of volume to contact area (conventionally 1 kg/6 dm^2). Assuming a reasonable effort has been made to develop a sensitive method, the use of a substitute test can be accepted. A typical example is an antioxidant, with a low SML, which cannot be isolated from the fat simulant to an acceptable level.

2.8.2 Directive 97/48/EC, Chapter 3, point 2, provides the following clause, which applies to overall and specific migration:

"By derogation ....., it may be possible to omit one or two of the substitute tests ..... if these tests are generally recognised not appropriate for the sample under consideration on the basis of scientific evidence".

Both overall migration and specific migration are influenced by the physical properties of the polymer, the migrant and the simulant. Without being exhaustive, the following parameters will influence migration into simulants and also mass transfer into test media:

- physical properties and polarity of the polymer;

- diffusivity of the migrant in the polymer;

- interaction of the simulant or the test medium with the polymer or with the migrants;

- time and temperature conditions of the test.

Penetration of the polymer by olive oil (or other non-volatile fatty simulants) can greatly accelerate the migration process. Therefore the interaction of substitute media with the material should be close or slightly greater than that of simulant D in the corresponding time and temperature conditions (Directive 97/48/EC, Table 4). However the determination of the interaction of the simulant or the test medium with the polymer may be complex. The affinity of a migrant with a food simulant can be reflected in some cases by its solubility in the simulant. In these cases mass transfer is expected to be low when the migrant is poorly soluble in the simulant or in the test medium. Therefore, comparison of the solubility (or solubility range) in simulant D and in test media in the corresponding time and temperature conditions (see Directive 97/48/EC, table 4) may be used as a first indication to help select the most suitable test medium.

For example: Ethanol 95% is a suitable test medium for non-polar polymers such as polyolefines and also for plastics of medium polarity like PVC and PET. However it is inappropriate for strong polar polymers (e.g. PA), for which iso-octane can be used if the limits are exceeded.

It is recommended that analysts maintain the comparison curves of simulant D and the test medium used in the alternative test. These curves should be requested by the national enforcement authorities as well as the Commission to verify the validity of the omission of a test medium. These comparison curves shall be always added in the petitions in order to have a Community agreement.

2.8.3 Test media

2.8.3.1 Iso-octane

The majority of published data demonstrate the suitabilityof iso-octane as a volatile test medium in the substitute fat test for the determination of overall migration. In EC Research project No 33 revision 1 entitled "Migration testing with conventional and alternative fatty-food simulants" an overview is presented of all comparative data, available at that time (Feb. 1996), using a fat simulant or a volatile test medium. The compilation can be used to indicate suitability of the chosen volatile test medium.

However it should be noted that some special types of polyolefinsmay give migration values with iso-octane higher of those expected in the real use. Also polystyrene containing more than 6.5% of polybutadiene and/or mineral oil may give high results, while polyamides may give low results.

Data on specific migration into iso-octane are more scarce and use of iso-octane in specific migration testing should therefore be considered on a case by case basis.

As regards the analytical procedure consult the document "Methods of Analysis".

2.8.3.2 Ethanol 95%

The previously mentioned EC study (EC Research project No 33, revision 1) also contains useful data about the use of 95% ethanol as a volatile test medium in substitute tests. Also in that case mainly overall migration data are available.

Usually it is found that a good comparison is obtained between olive oil and 95% ethanol. It is found that 95% ethanol is more suitable for testing polystyrene/butadiene blends than iso-octane. However, it is also the case that values obtained with 95% ethanol tend to give slightly lower results compared to olive oil for most polyolefins.

Data on specific migration into 95% ethanol are relatively scarce and use of 95% ethanol in specific migration testing should therefore be considered on a case by case basis.

As regards the analytical procedure consult the document "Methods of Analysis".

2.8.3.3 Modified polyphenylene oxide (MPPO)

In order to circumvent a number of analytical difficulties caused by the overall migration determination with food simulant D at high temperatures another test has been developed using MPPO as an absorption test medium. The draft Directive specifies the conditions to be fulfilled before applying this test i.e.:

a) the contact temperature in worst foreseeable use is higher than 70°C;

b) the results obtained in a "comparison test" are equal to or greater than those obtained in the test or with the abovementioned substitute tests or with representative foodstuffs;

c) the migration limits are not exceeded.

Comparative studies with ^{14}C-labelled HB307 have shown that in experiments with a polypropylene and a polyetherimid tray at test conditions of 2 hours/100°C and 2 hours/175°C, respectively, the MPPO adsorbed amount is equivalent to overall migration in fat simulant D. In addition, further studies with a number of organic substances have shown that MPPO provides stronger adsorption capacities than real foodstuffs such as pizza, pastry etc.

2.8.3.4 Other substitute media

The cases in which both substitute tests are recognised to be unfeasible for technical reasons connected with the method of analysis should be very rare. However in order to give legal guidance in every possible situation, the text offers the possibility to use other media e.g. MPPO or isopropanol (this last should be used at the same conditions of ethanol 95%).

2.9 Alternative tests

2.9.1 Directive 97/48/EC in Chapter 4, point 1, provides the following clause;

"It is permissible to use the result of alternative tests as specified in this Chapter provided that both the following conditions are fulfilled:

a) the results obtained in a "comparison test" show that the value are equal to or greater than those obtained in the test with simulant D;

b) the migration in alternative test does not exceed the migration limits, after application of appropriate reduction factors provided in Directive 85/572/EEC.

If either or both conditions are not fulfilled, then the migration tests must be performed."

Regarding tests with fatty food simulant, if certain specified conditions are satisfied the Directive allows the possibility of replacing the tests described in Chapter 1, 2 and 3 either by alternative tests using volatile media, for example iso-octane or ethanol 95%, or by "extraction tests" which are tests with very aggressive volatile solvents used at high temperature.

The Directive does not specify how equivalency or greater severity of the alternative tests should be demonstrated in practice. In practice the frequency will depend on the particular situation under examination. If the alternative tests give the values of the released substances higher than those obtained by simulant D, it is not necessary to frequently repeat the comparison tests (check), provided the process of manufacture ensures a high probability that the reproducibility of the characteristics of the final article are constant. In this situation a check once each year could be sufficient. The check should be repeated more frequently if these conditions are not satisfied.

It should be emphasised that as the alternative tests are conventionally deemed equivalent to or giving higher values than the tests using simulants D, the reduction factors also apply to the alternative tests.

2.9.2 "Alternative test with volatile media"

The Directive does not specify the type of volatile test medium to be used as an alternative to the simulant D and the test conditions to be used. In fact it is impossible to establish a general relationship between the test conditions of simulant D and the alternative volatile test medium.

Therefore each analyst should select the appropriate alternative volatile medium taking into account the general considerations mentioned in point 2.8.2. and construct for each polymer the migration curves (migration against time for the different temperatures prescribed by the Directive according to rules of Chapter 2). From these curves the test conditions to be used with the alternative test medium should be selected in order to obtain the same results or better, migration values higher than

those obtained by simulant D. It is recommended that the choice of test conditions for the alternative volatile medium is such that there is a sufficient margin of the security between the values obtained with olive oil and those (higher) obtained with the volatile medium.

2.9.3 Extraction test

Directive 97/48/EC, Chapter 4, point 3.2, provides the following clause:

"Other tests, which use media having a very strong extraction power under very severe test conditions, may be used if it is generally recognised, on the basis of scientific evidence, that the results obtained using these tests ("extraction tests") are equal to or higher than those obtained in the test with simulant D."

On this basis, rapid extraction tests using appropriate solvents such as diethelether, iso-octane, ethanol 95% have been developed. A strong interaction with the polymer is achieved and, as a consequence, a rapid extraction test is obtained. This allows the amount of potential migrants to be determined which, in general, is higher than the migration into food simulants. These extraction tests are most suitable for the overall migration assessment of flexible packaging plastics with a thickness less than or equal to 300 µm. Appropriate test media were found it be iso-octane for non-polar plastics like polyolefines and 95% ethanol for more polar plastics such as polyamide. In case of doubt, both test media should be applied and the higher result used. Suitable test conditions were found to be 24 hours at 40°C. The method can also be applied to thicker materials provided the overall migration limit of 10 mg/dm^2 is not exceeded.

The method may also be suitable for specific migration assessment if it can be demonstrated that it achieves almost complete extraction from a polymer to calculate then the maximum possible migration under the assumption of total mass transfer.

Consult also the document "Methods of analysis".

3. Calculation of the maximum possible migration

Maximum possible migration can be calculated on the basis of residual or actual content of the migrant in the polymer sample. For that purpose the content of migrant in the polymer has to be determined by e.g. exhaustive extraction or dissolution of the polymer. This procedure has the advantage that the results can be easily extrapolated to any other food contact article made of the same polymer, with only one test having to be performed.

This calculation is made by applying the following formula:

$$M = \frac{Q \times A \times Lp \times D}{\_\_\_\_\_}$$

1000

where:

M = is the maximum possible migration of the substance expressed in mg/kg foodstuff or mg/6 dm^2 of food contact material.

Q = is the quantity of the substance in the finished article in mg/kg polymer.

A = is area of the food contact material in cm^2, conventionally set at 600 cm^2.

Lp = is thickness of the food contact material in cm.

Maximum thickness can be set at 0.025 cm which conventionally is assumed to give maximum migration with the exception of plasticised polymers and of the migration of components with low diffusion coefficients (volatile components).

D = is density of the polymer in g/cm^3.

For example:

The residual content of migrant X in a polyethylene with a density of 0.95(g/cm^3) has been determined to be 4.5 mg/kg polymer. The food contact material is used in a wide range of materials with a maximum thickness of 0.018 cm. Then M = the maximum migration may be:

$$M = \frac{4.5 \times 600 \times 0.018 \times 0.95}{1000} = 0.046 \text{ mg/kg foodstuff}$$

4. Exemptions from migration testing

Migration tests can be avoided, for example, in the following circumstances:

a) when the specific limit for the substances to be checked is higher than the overall migration level found in the experiments or by calculation (see Directive 90/128/EEC, article 5, point 2);

b) when assuming a 100% migration (see point 3) the limit(s) cannot be exceeded in any foreseeable conditions[9];

c) when a test may be considered less severe than another according to the criteria established in the EC Directives or in this document;

[9] In this case the petitioner should provide an adequate method for the analysis of the substance in the finished product

d) when it can be demonstrated by generally recognised diffusion models that the amount of substance in the material is such that the limit(s) cannot be exceeded in any foreseeable conditions[xii].

5. Labelling related to the migration testing

Directive 97/48/EC, Chapter 1, point 2.2c, provides the obligation of an appropriate indication when a material or article is intended to be or not to be in contact with some foodstuffs or groups of foodstuffs. According to this rule

'This indication shall be expressed:

(i) at the marketing stages other than retail stage, by using the "reference number" or "description of foodstuffs" provided in the Table of the Directive 85/572/EEC;

(ii) at the retail stage using an indication which shall refer to only a few foods or groups of food, preferably with examples which are easy to understand."

It is known that some films are unable to comply with the migration limits in the fat test unless a reduction factor is applicable. Conventionally called "factor X film", a film which complies with the migration limits in the fat test after the reduction factor equal to X is applied. For instance a film having an overall migration in the fat test of 20 mg/dm^2 is in compliance with the overall migration limit at 10 only if the foodstuffs in contact have a reduction factor of 2. This film is called "Factor 2 film".

These films, which could be sold as such in supermarkets etc. should be labelled adequately in order to exclude the possibility that consumers might place the film in contact with food or groups of foods not having the appropriate reduction factor.

Some examples of suitable labelling are suggested below:

5.1 Factor 2 films

"Suitable for contact with all foodstuffs except pure fats and oils, and food preserved in an oil medium."

5.2 Factor 3 films

"Suitable for contact with all foodstuffs except pure fats and oils, butter and margarine and food preserved in an oil medium."

5.3 Factor 4 films

"Suitable only for foodstuffs of which the following are examples:

Fresh meat and poultry

Processed meat products

Fried or roasted foods

Fruit and vegetables

Frozen foodstuffs

Bakers products and solid confectionery"

Annex 1

GUIDELINES FOR THE DESCRIPTION OF THE METHODS OF ANALYSIS

As stated in "SCF-WG Explanatory Guidance", a method of analysis must be included in the technical dossier. In order to help the applicant, some general indications are given below. However it is recommended to follow, as much as possible, the format recently adopted at CEN level, which is also reported later.

Methods should be capable of either quantification of the substance in the material or article itself or quantification in appropriate food simulants (or foods) respectively.

Method of analysis should comply with the following format (specimen examples may be seen in EN Methods for Food Contact Materials (See also the document "Methods of Analysis")

1. Scope
2. Principle
3. Sampling
4. Reagents (Safety precautions)
5. Apparatus
6. Procedure
7. Confirmation
8. Precision
9. Test report

1. SCOPE

   Statement of types of materials and articles for which the method is applicable. Statement of food simulants (or foods) for which the method is suitable. Statement of the limit for which the method is capable of quantitative determination of the substance in the material and article or food simulant (or food).

2. PRINCIPLE

   Statement of the principle that is employed for the determination (for example headspace GC, extraction followed by HPLC, extraction followed by colorimetric determination).

3. REAGENTS

Statement of safety requirements and any special precautions in handling reagents. Statement of purity requirements of substance (obtainable from EC-JRC)[10], internal standard and any special requirements for solvent or reagent purity. Statement of primary and diluted calibrant solutions which should have a concentration range to span the QM or SML limit.

4. APPARATUS

Normal laboratory apparatus can be assumed but any instrument or special piece of apparatus or particular specification should be stated.

The minimum performance of chromatographic methods should be stated in terms of the resolution of the substance to be determined from internal standard, solvent or other components. Examples of columns found to be suitable should be given.

5. SAMPLES

Statement of requirements for taking of representative samples of materials and articles for analysis. For testing with simulants the guide to the selection of conditions and methods of test is stipulated in an EN Method (see the document "Methods of analysis").

6. PROCEDURE

Statement in sufficient detail of how to carry out procedure which should include the manner of preparation of calibration curves, evaluation of data, and final determination graphically or by calculation.

As quantitative extraction from materials and articles can never be fully demonstrated the method of standard addition should always be employed for calibration. For determinations of substances in food simulants an internal standard should always be employed for chromatographic procedures and calibration should be against blank food simulant fortified with the substance in question.

7. CONFIRMATION

The method of analysis must include details for confirmation of test results to be used in cases where the measured QM or SML values have been found to exceed the limits specified in Directive 90/128/EEC and subsequent amendments.

The principle behind the confirmation step is that the technique used is sufficiently different from that first used, that it confers additional assurance of identity and level of putative substance. Thus for example :

10 See "Eu and national authorities"

For volatile substances where GC is employed then confirmation by GV/MS (scanning or selected ion monitoring) is appropriate polarity or derivative formation. For non-volatile substances using HPLC, confirmation can be carried out by GC/MS after formation of a suitable volatile derivative or by using at least one other HPLC column with differing separation characteristics and a different solvent system, and/or stopped-flow scanning UV or fluorescence studies.

8. PRECISION

Statement of the detection limit of the method of analysis and the limit of quantification. The analytical tolerance that will be applied to QM or SML limits will depend on the performance of the method and the calculation of a critical difference value that can only be obtained by inter-laboratory collaborative trial. However, the method should include a statement of the within-laboratory "repeatability" of the method obtained by the proposer of the method or similar laboratory.

9. TEST REPORT

The test report should give the relevant information on the method used .

(extract from CEN document, final version - 18 March 1992)

STANDARD FORMAT FOR DRAFTING OF CEN METHODS FOR DETERMINATION OF PLASTICS CONSTITUENTS IN FOODSTUFFS, FOOD SIMULANTS AND MATERIALS AND ARTICLES

PART 0. EXPLANATORY NOTE

This Standard Format has been prepared by Task Group 4 of Working Group 5 of CEN TC194 'Utensils in contact with food' as a template for drafting analytical methods of test for plastics materials and articles destined to come into contact with foodstuffs.

The analytical methods of test are concerned with the determination of specific migration of plastics constituents into foodstuffs and food simulants and with the determination of residual constituents in plastics materials and articles.

The Standard Format consists of two parts:
Part 1. STANDARD FORMAT sets out the minimum requirement of items to be covered in the description of an analytical method of test. The items are given in a very general way only.

Part 2. GUIDELINE FOR COMPLETION OF STANDARD FORMAT sets out in what way the items in Part 1. can be elaborated in a particular case in order to obtain the full description of the method.

Therefore Part 1 should be read in direct conjunction with Part 2.

1. **STANDARD FORMAT**

TC194/[PM/REF-Y]
[ISSUED]

MATERIALS AND ARTICLES IN CONTACT WITH FOODSTUFFS

PLASTICS

PART [X]. DETERMINATION OF [ANALYTE] IN [MATRIX]

WARNING: [SET OUT]

Contents

FOREWORD

This part of European Standard EN [XXX] has been prepared by Working Group 5 of TC194 'Utensils in contact with food' as one of a series of analytical methods of test for plastics materials and articles intended to come into contact with foodstuffs.

The methods of test are concerned with the determination of overall and specific migration of plastics constituents into foodstuffs or food simulants and with the determination of residual content of plastics constituents in the finished plastics product.

[ANNEX]

This part should be read in conjunction with PART 1 of EN [XXX].

0. INTRODUCTION

ANALYTE, FORM], [PM/REF] is a [CONSTITUENT] used in the manufacture of certain plastics materials and articles intended to come into contact with foodstuffs.
After the manufacture residual [ANALYTE] can remain in the finished product and may migrate into foodstuffs coming into contact with that product.

The method described in this European standard allows of the determination of [ANALYTE] in [MATRIX]. The method is to be used in conjunction with Part 1 of EN [XXX] which describes the procedures required prior to the determination of [ANALYTE] in [MATRIX].

The method has been validated by collaborative trial using [MATRIX] (see 8).

1. SCOPE

This part of EN [XXX] describes a method for the determination of [ANALYTE] in [MATRIX].

The method is appropriate for the quantitative determination of [ANALYTE] in approximate analyte concentration range of [RANGE] [MASS]/kg [MATRIX].

2. PRINCIPLE

The level of [ANALYTE] in [MATRIX] is determined by [TECHNIQUE]. Quantification is achieved using [STANDARD].

[CONFIRM].

3. REAGENTS

Reagents and solvents shall be of analytical quality.

3.1 [ANALYTE ST, FORM]
(PURITY)

3.2 [STANDARD, FORM]
(PURITY)

3.3 [REAGENTS]
(PURITY)

3.4 [SOLUTIONS]
(INSTRUCTIONS)
(CONDITIONS)

4. APPARATUS

NOTE An instrument or item of apparatus is listed only where it is special or made to a particular specification, usual laboratory glassware and equipment being assumed to be available.

4.1 [SPECIAL]

5. SAMPLES

The laboratory samples of [MATRIX] to be analysed are obtained as described in PART 1 of EN [XXX]. Analyte-free samples of [MATRIX] of the same type as those to be analysed are also required for use for calibration purposes.

(CONDITIONS)

5.1 Test sample preparation

(DESCRIPTION)

5.2 Calibration sample preparation

(DESCRIPTION)

5.3 Blank sample preparation

(DESCRIPTION)

6. PROCEDURE

6.1 [TECHNIQUE] parameters

(DESCRIPTION)

6.2 Optimisation of instrumentation

(DESCRIPTION)

6.3 Calibration

(DESCRIPTION)

6.4 Execution of determination

(DESCRIPTION)

6.5 Evaluation of data

NOTE The following calculations assume that for all measurements exactly the same weight or volume of [MATRIX] has been used and, for the internal standard, that invariably the same volume of internal standard solution has been added.

6.5.1 [TECHNIQUE] interferences

(DESCRIPTION)

6.5.2 Calculation of analyte level

(DESCRIPTION)

7. CONFIRMATION

In cases where [SML or QM] of [ANALYTE], calculated according to the procedure given in Part 1 of EN [XXX] from the analyte level calculated according to Section 6.5 exceeds the restriction criterion set in Commission Directive 90/128/EEC (SML or QM), the result of the determination shall be confirmed. The confirmation is qualitative in the sense that it should demonstrate the correct identity of the measured analyte and the absence of interferences. For the purposes of quantitation the result as calculated according to Section 6.5 shall be taken as the true value.

(DESCRIPTION)

8. PRECISION

Method performance has been evaluated by carrying out a precision experiment according to ISO 5725-1990 'Accuracy (Trueness and Precision) of Measurement Methods and Results', Parts 1-6.

8.1 Validation *(N.B. For the applicant this item may be omitted)*.

For validation of this method a precision experiment was conducted in [YEAR], involving [NUMBER] laboratories. Each participant in this experiment obtained [NUMBER] samples of [ANALYTE]-free [MATRIX] together with sets of [NUMBER] samples of [MATRIX] fortified with [ANALYTE] at levels of approx. [LEVEL] [MASS]/kg respectively.

Calibration solutions were prepared with comparable concentrations so that the calibration samples could be corrected.

8.2 Repeatability and reproducibility

Evaluation of the results of the precision experiment at a concentration of [LEVEL] [MASS] [ANALYTE]/kg [MATRIX] for the 95% probability level yielded the following performance characteristics:

Repeatability: r = [LEVEL][MASS][ANALYTE]/kg

Reproducibility: R=[LEVEL][MASS][ANALYTE]/kg *(N.B. For the applicant this item may be omitted).*

There was no influence of the calibration method using [STANDARD] on the numerical values of r and R.

8.3 [LIMIT]

The [LIMIT] of [ANALYTE] - measured as equal to the mean content of representative [BLANK] (n _ 20) plus three times the standard deviation of the mean - was found to be in the range of [RANGE] [MASS] [ANALYTE]/kg [MATRIX].
Thus the method is capable of quantitative determination of [ANALYTE] at a minimum level of [LEVEL] [MASS]/kg [MATRIX].

8.4 Critical [ANALYTE] level

The question whether there is a significant difference for the 95% probability level between the restriction for [ANALYTE] - i.e. [RESTRICTION] - and [SML or QM], calculated from the analyte concentration in [MATRIX] determined by this method, can be decided by means of the critical difference CrD_{95}.

If the determined [ANALYTE] level in [MATRIX] exceeds the limit value calculated from the [RESTRICTION] by more than CrD_{95}, [SML or QM] of [ANALYTE] must be considered to exceed the [RESTRICTION].

So, if analyte level and CrD_{95} are expressed in mg/kg [MATRIX]:

Critical [ANALYTE] level = [RESTRICTION] + CrD_{95} mg/kg [MATRIX].

Evaluation of the results obtained in a precision experiment involving [NUMBER] laboratories resulted in:

CrD_{95} = [LEVEL] [MASS]/kg [MATRIX].

9. TEST REPORT

The test report shall contain, as a minimum, the following:

- an identification
- name of laboratory
- name of person responsible for analysis
- date of report
- date of analysis
- analyte
- a reference to this method
- performance characteristics of the method
- sample details, such as:

    - type of food/food simulant/material/article
    - origin and denotation of the sample
    - date and method of obtaining the laboratory sample
    - storage conditions

- results expressed in [MASS] [ANALYTE]/kg [MATRIX]. Results should be reported as the average value from two or more determinations satisfying the repeatability criterion of Section 8.2
- details of confirmation procedure, if any
- reasons for modifications introduced into the test method, if any.

2. GUIDELINE FOR COMPLETION OF STANDARD FORMAT

Expressions between brackets in PART 1. STANDARD FORMAT should be completed as follows:

Method No.:

[PM/REF-Y] = set out EEC PM/REF No. of analyte and Y = version no. of method.

Date of issue:

[ISSUED]= set out month (abbreviated) and year of issue, e.g. 'Feb. 1993'.

PART No.:

[X] = set out part no. of method in European Standard [XXX].

PAGE No.:

[page p of q] = set out p = sequential number of page and q = total number of pages of method description.

Throughout PART 1. STANDARD FORMAT:

[XXX] = number of European Standard
[ANALYTE] = set out food contact material constituent to be determined
[MATRIX] = set out foods and/or food simulants in which food contact material constituent can be determined by this method.

WARNING:

[SET OUT] = set out whether analyte or any other chemical involved in the procedure is hazardous or harmful to health and what precautions must be taken before or during application of the method.

Contents:

[ANNEX] = set out annexes, if any

FOREWORD:

[ANNEX], if any = set out 'Annex to this standard is normative, where applicable'.

0 INTRODUCTION:

[ANALYTE, FORM] =	set out analyte to be determined, bruto formula inclusive
[PM/REF] =	set out EEC PM/REF No. of analyte
[CONSTITUENT] =	set out 'monomer' or 'additive' or 'aid to polymerisation'.

1. SCOPE:

[RANGE] =	set out numerical values of analyte concentration range
[MASS] =	set out 'µg' or 'mg'.

2. PRINCIPLE:

[TECHNIQUE] =	set out principle of method used to determine analyte in matrix, e.g. 'headspace gas chromatography' or 'solvent extraction, then gas chromatography' or 'high performance liquid chromatography', etc.
[STANDARD] =	set out whether an internal standard or an external standard is used or whether standard addition is applied.
Note 1:	An internal standard should be used whenever possible and an explanation should be offered for not using one.
[CONFIRM] =	set out what confirmation procedure is used.

3. REAGENTS:

3.1	[ANALYTE ST, FORM]=	set out analyte standard used for calibration, bruto formula inclusive
	(PURITY) =	set out purity requirements, if any, of analyte standard.
3.2	[STANDARD, FORM]=	set out internal or external standard, bruto formula inclusive
	(PURITY) =	set out purity requirements, if any, of internal standard, if any.
	Note 2:	In general the internal standard should contain no impurity at > 1% by peak area or peak height which will elute at the same retention time as that of the analyte.

3.3 [REAGENTS] = set out chemicals, other than analyte standard and internal standard or external standard and solvents involved in the procedure

(PURITY)= set out purity requirements, if any, of reagents and solvents, or set out 'all reagents shall be of analytical quality'.

3.4 [SOLUTIONS]= set out solutions, concentration inclusive, involved in the procedure, such as:

- primary solution of analyte standard
- dilute solution(s) of analyte standard
- solution(s) of internal or external standard
- mobile phase
- reagent solutions
- etc.

(INSTRUCTIONS) = set out detailed instructions for preparation of solutions.

Note 3: Two primary solutions of analyte standard should be prepared and checked against one another with one dilution only. Ifthere is agreement within 5% then further dilute standards are made from only one of the primary standards.

Note 4: Avoid weights of analyte and internal standard larger than 150 mg and also avoid volumes of solvent greater than 100 ml.

(CONDITIONS)= set out conditions of storage and maximum storage time for solutions, as obtained from stability tests.

4. APPARATUS:

4.1 [SPECIAL] = set out special equipment e.g.:

- gas chromatograph, equipped with:
  - automatic headspace sampler
  - alkali flame-ionisation detector
- chromatographic column
- etc.

Note 5: set out column requirements, such as:

- the column must exhibit reasonable peak shape with respect to half-width and asymmetry and must permit the separation of analyte and internal standard

- the column must exhibit minimum overlap of peaks of analyte and internal standard and other substances. A check should be specifically carried out on interference with the internal standard.
- etc.

Note 6: set out examples of columns that have been found suitable for analyte determination - include details of type, dimensions, column flow, temperature etc.

5. SAMPLES:

(CONDITIONS) = set out conditions of storage of samples

Note 7: Analytical determinations should be carried out on duplicate samples, these being duplicate portions of [MATRIX], with at least duplicate measurements (injections) of the final extract.

5.1 Test sample preparation:

(DESCRIPTION) = set out test sample preparation.

5.2 Calibration sample preparation:

(DESCRIPTION) = set out calibration sample preparation.

5.3 Blank sample preparation:

(DESCRIPTION) = set out blank sample preparation.

6. PROCEDURE:

6.1 [TECHNIQUE] parameters:

[TECHNIQUE] = set out 'GC' or 'HSGC' or 'HPLC', etc.

(DESCRIPTION)= set out established parameters or guidance parameters, e.g. injector/column/detector temperature, carrier gas and flow rate, etc.

6.2 Optimisation of instrumentation:

(DESCRIPTION) = set out optimisation of instrumentation.

Note 8: For methods involving GC or HPLC, optimisation will be required in terms of demonstrating adequate specificity and sensitivity. The satisfactory choice of

column should be demonstrated, and optimum instrumental parameters should be established, such as:

- injector temperature
- column temperature
- detector voltage/wavelength
- detector temperature
- detector gas flow rate(s)
- carrier gas/elution solvent
- carrier gas flow rate/elution solvent flow rate
- etc.

Some indication should be given of the minimum requirement in terms of detector performance, e.g.: should be able to detect 20 pg on-column of analyte at a signal to noise ratio of 5:1.

6.3 Calibration:

(DESCRIPTION) = set out in what way calibration is achieved.

i. By calibration graph using an internal or external standard:

- the calibration graph shall be constructed from at least five measurements
- concentration range of analyte calibration solutions shall span from x 0.1 specific migration limit (SML) or x0.1 residual content limit (QM) to x 2.0 SML or x 2.0 QM
- the calibration graph shall be rectilinear
- the correlation coefficient shall be 0.996 or better.

Set out construction of calibration graph.

ii. By calibration graph employing standard addition:

- the sample with no addition of analyte standard solution shall be analysed in triplicate
- addition of analyte standard shall be at three levels, i.e. at sample level, at double and at thrice the sample level
- analyses shall be carried out with at least duplicate measurements (injections) of the final extracts
- the standard addition graph shall be rectilinear
- the standard error on the intercept shall not exceed a coefficient of variation of 10% of the mean value.

Set out construction of calibration graph.

iii. Where recovery experiments are appropriate (e.g. with methods involving extraction, without standardisation and not using standard addition) they shall be carried out in duplicate, using at least three different analyte concentrations. Where correction for recovery is appropriate recovery shall be 70% or better.

Set out recovery experiments.

6.4 Execution of determination:

(DESCRIPTION) = set out execution of the determination.

6.5 Evaluation of data:

6.5.1 [TECHNIQUE] interferences:

[TECHNIQUE] = set out 'GC' or 'HSGC' or 'HPLC', etc.
(DESCRIPTION) = set out possible interferences and set out instructions to solve the problems.

6.5.2 Calculation of analyte level:

(DESCRIPTION) = set out in what manner analyte level in the matrix is calculated.

Note 9: Either a mathematical or a graphical method may be applied to calculate analyte level in the matrix.

7. CONFIRMATION:

[SM or QM] = set out 'specific migration' or 'residual content'

(DESCRIPTION) = set out in what way confirmation is achieved, e.g.:

i. For volatile substances, determined before by a GC-procedure:

i.1 Using gas chromatography/mass spectrometry (GC/MS):

Note 10: If the SML or QM for the analyte and the method allow for more than 20 ng analyte/injection then full mass scanning should be carried out for the supposed analyte peak, looking for a correspondence in the analyte spectrum and in the spectrum of the analyte standard, in terms of presence and correspondence of relative intensities of specified characteristic ions.

If the analyte mass is estimated to be less than 20 ng/injection then the selected ion monitoring (SIM) mode should be used. Confirmation is now achieved by observance of the presence of two characteristic ions - one of those for preference being the molecular ion - at the retention time of the analyte, which in relative abundances agree to ± 10%.

NOTA BENE: SIM conditions could also be stated for quantitative confirmation.

Set out in what way confirmation of determination is carried out.

i.2 Using at least one other column with a different polarity:

Note 11: A peak must be found at the correct retention time for analyte ± 3%, and when measured the quantitative result for the two columns must agree to within ± 10%, or - if within less than 10% - within ± the critical difference CrD95 for the method.

Set out in what way confirmation of determination is carried out.

ii. For non-volatile substances, determined before by anHPLC-procedure:

ii.1 By formation of a volatile derivative:

Note 12: qualitative confirmation may be obtained by formation of a volatile derivative which subsequently is examined by GC/MS as described in Section i.1.

Set out in what way confirmation of determination is carried out.

ii.2 By formation of a non-volatile derivative:

Note 13: Qualitative confirmation may be obtained by formation of a non-volatile derivative which subsequently is subjected to HPLC examination. The shift in retention time as compared to that of the analyte must be found to correspond to within ± 3% with the shift in retention time obtained for the analyte standard.

Set out in what way confirmation of determination is carried out.

ii.3 Using at least one other column with differing separation characteristics and a different solvent system:

Note 14: A peak must be found at the correct retention time for analyte ± 3%, and when measured the quantitative result for the two columns must agree to within ± 10%, or - if within less than 10% - to within ± the critical difference CrD95 of the determination.

Set out in what way confirmation of determination is carried out.

ii.4 Using a UV or diode array detector:

Note 15: When using a UV detector, absorbance values for analyte at three separate wavelenghts should agree to within ± 3% with that of the analyte standard. When using a diode array detector, correspondence of spectra of analyte and analyte standard should be obtained.

Set out in what way confirmation of determination is carried out.

8. PRECISION:

8.1 Validation *(N.B. For the applicant this item may be omitted)*.
:

[YEAR] = set out year in which precision experiment was performed
[NUMBER] = set out number of laboratories or number of samples
[LEVEL] = set out numerical values of levels of analyte
[MASS] = set out 'µg' or 'mg'.

8.2 Repeatability and reproducibility (N.B. For the applicant the reprodicibility may be omitted):
:

[LEVEL] = set out numerical value of level of analyte
[MASS] = set out 'µg' or 'mg
[STANDARD] = set out 'internal standard' or 'external standard' or 'standard addition'.

8.3 [LIMIT]:

[LIMIT] = set out 'detection limit' or 'determination limit'
[BLANK] = set out 'matrix blanks' or 'matrix blanks fortified with analyte at the level of x 0.1 SML' or 'matrix blanks fortified with analyte at the level of x 0.1 QM'

[RANGE] = set out numerical values of analyte concentration range
[MASS] = set out 'µg' or 'mg'
[LEVEL] = set out numerical value of level of analyte.

8.4 Critical [ANALYTE] level:

[RESTRICTION]= set out 'SML' or 'QM' or a value derived from one of either of those
[SM or Q] = set out 'specific migration' or 'residual content'.
[LEVEL] = set out numerical value of level of analyte.
[MASS] = set out 'µg' or 'mg'.

9. TEST REPORT:

[MASS] = set out 'µg' or 'mg'.

Annex 2

EXPLANATORY NOTE" ON THE USE OF t-T TABLE IN DIRECTIVE 82/711/EEC AS AMENDED FOR SELECTION OF CONDITIONS IN MIGRATION TESTING

(called briefly "Guidelines for Selection of Test Conditions")

NOTA BENE: This appendix was prepared by TNO under EC contract.

Introduction

It has already become evident that the text of Chapter II of Directive 93/8/EEC needs some elucidation in order to make interested parties better understand how to select conditions in migration testing (simulant, time and temperature) that would match conditions of contact between foodstuffs and food contact materials in practice.

In order to meet this need an inventory was drawn up in the first place by the Commission of conditions of contact between foods and food contact materials practised in the food industry in preparing and storage of packed foods.
Subsequently the data collected was entered into a table which is similar to the one in Directive 85/572/EEC for the classification of foodstuffs.
The contact time in actual use, as presented in the table, are assumed to represent maximum contact time.

In the table conditions for testing such as food simulant, time and temperature are proposed that match the conditions of contact in practice of food and food contact material.

In case of multi-layer materials the contact material is mentioned first (e.g. paper/alufilm). In many cases the packaging consists of various parts that all are in contact with the food (e.g. plastic film/plastic tray). In that case each part should be subject to migration testing.
For the sake of completeness, packaging materials, like glass or coated board, are included which are not yet covered by an EC Directive. These materials are not yet subject to migration testing.

It goes without saying that the list of packed foods in the table is not complete. Interested parties are invited to provide the Commission with additional information which would allow the table to be corrected where necessary and to enlarge the number of examples. Coöperation of several experts in food industry as well as of retail store managers in drafting the guidelines is gratefully acknowledged.

Explanation of expressions and abbreviations

dfr	deep-freezer
h	hour
lam	laminate
m	month
mw	microwave
past	pasteurized
pl	plastic
ref	refrigerator (3 - 10°C)
ster	sterilized

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
01.	**Beverages**					
01.01	non-alcoholic beverages or alcoholic beverages of an alcoholic strength lower than 5 % vol.:					
	beer, various	can		X		0.5h -pas ambient s
	fruit juice, various	coated board				0.5 h pas ambient
	fruit juice, various	can		X		0.5h past
	lemon juice	pl bottle/pl closure		X		>1y-ambi
	lemonade syrup	pl bottle/pl closure		X		1y -ambi
	mineral water	glass bottle/pl closure	X			1y -ambi
	mineral water	coated board	X			1m -ambi
	soft drinks, various	can		X		1y -ambi
	soft drinks, various	pl bottle/pl closure		X		1y -ambi
01.02	alcoholic drinks of an alcoholic strenght equal to or exceeding 5% vol.:					
	beer, various	glass bottle/pl closure		X	X	0.5h pas
	alcoholic drinks	glass bottle/pl closure		X 2)	X 3)	>1y-ambi
	egg-and-brandy liqueur	glass bottle/pl closure			X 3)	>1y-ambi
01.03	miscellaneous: non-denatured ethanol					
02.	**cereals, cereal products, pastry, biscuits, cakes and other baker's ware**					
02.01	Starch					
02.02	cereals, unprocessed, puffed and in flakes, including popcorn, corn flakes and the like					
	Muesli	pl sachet				6m -ambi
	Popcorn	pl bag				1y -ambi
	Rice	pl bag				>1y-ambi
02.03	cereal flour and meal					
02.04	pasta (macaroni, spaghetti, vermicelli, etc.)					
	spaghetti, macaroni, etc.	pl bag				1y -ambi
02.05	pastry, biscuits, cake and other dry baker's ware A. with fatty substances on the surface					
	biscuits, various	pl tray/pl film			X/5	1y -ambi
	Cake	pl tray/pl film			X/5	3m -ambi

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
	cake base	pl tray/pl film			X/5	6m -ambi
	cheese crackers	pl film			X/5	1y -ambi
	fancy cakes	pl tray/pl film			X/5	3m -ambi
	pastry	pl film			X/5	1m -ambi
	pastry, various	pl film/pl tray			X/5	1m -ambi
	salty biscuits, various	pl film			X/5	6m -ambi
	sand cakes	pl tray/pl film			X/5	6m -ambi
	spicy biscuits	pe/paper lam			X/5	1m -ambi
	treacle wafers	pl film			X/5	3m -ambi
	B. other					
	biscuits, various	pl film				6m -ambi
	cocos bread	pl film	X			6m -ambi
	instant bread	pl bag				3m -ambi
	reform biscuits	pl tray/pl film				6m -ambi
	rusk	paper + pl film				6m -ambi
02.06	pastry, cake and other fresh baker's ware					
	A. with fatty substances on the surface					
	bread, various	pl bag			X/5	1w -ambi
	buns	pl film			X/5	1w -ambi
	coffee rolls	pl bag			X/5	1m -ambi
	fancy pastries, various	pl film/pl tray			X/5	1w -ref
	B. other					
	flans, various	pl film/pl tray	X			1w -ref
	gingerbread	pl film	X			3m -ambi
	pastry, various	pl tray/pl film	X			1m -ambi
	puffs	pl tray	X			1w -ref
	sugar bread	pl bag	X			1m -ambi
03.	**chocolate, sugar and products thereof, confectionery**					
03.01	chocolate, chocolate-coated products, substitutes and products coated with substi tutes					
	chocolate bars, various	paper/alufilm			X/5	6m -ambi
	chocolate granules	pl sachet			X/5	1y -ambi
	pralines	pl tray			X/5	6m -ambi
03.02	confectionery					
	A. in solid form					
	I. with fatty substances on the surface					
	II. other					
	marsh mellows	pl bag				1y -ambi
	sugared caraway seeds	pl sachet				1y -ambi
	sweets, various	pl sachet				1y -ambi

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
	B. in paste form:					
	I. with fatty substances on the surface					
	II. moist					
03.03	sugar and sugar-based products					
	A. in solid form					
	moist sugar	pl bag	X			>1y-ambi
	sugar	paper				1y -ambi
	B. honey and the like					
	honey	glass pot/pl closure	X			1y -ambi
	C. molasses and sugar syrup					
04.	**fruit, vegetables and products thereof**					
04.01	whole fruit, fresh or chilled					
	apples	pl bag				1w -ambi
	citrus fruit	pl bag				1w -ambi
	citrus fruit	pl net				1w -ambi
	cucumber	pl film				1w -ambi
	grapes	pl bag				1w -ambi
	passion fruit	pl box				1w -ambi
	strawberries	pl box				1w -ambi
04.02	processed fruit					
	A. dried or dehydrated fruit, whole or in the form of flour or powder					
	fruit snacks	can				>1y-ambi
	subtropical fruit	pl sachet				1y -ambi
	sultanas, various	pl sachet				1y -ambi
	B. fruit in the form of chunks, purée or paste					
	apple sauce	glass pot/twist-off cap		X		1h -ster
	apple sauce	can		X		1h -ster
	C. fruit preserves (jams and similar products - whole fruit or fruit chunks, flour or powder, preserved in a liquid medium):					
	I. in an aqueous medium					
	jams, various	glass pot/twist-off cap	X(a)	X(a)		hot fill >1y-ambi
						hot fill ambient
	olives	pl sachet	X			>1y-ambi
	II. in an oily medium					
	III. in an alcoholic medium (≥ 5% vol.)					

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
04.03	nuts (peanuts, chestnuts, almonds, hazelnuts, walnuts, pine kernels and other):					
	A. shelled and dried					
	nut chips, various	pl sachet				1y -ambi
	B. shelled and roasted					
	nuts, various	pl sachet			X/5 6)	6m -ambi
	C. in the form of paste of cream					
	hazelnut cream	glass pot/pl closure	X		X/5	1y -ambi
	peanut butter	glass pot/pl closure			X/3	1y -ambi
04.04	whole vegetables and potatoes, fresh or chilled					
	aubergines	pl film				1w -ambi
	beetroot	pl film				1w -ref
	broccoli	pl film				1w -ambi
	cabbage, various	pl film				1w -ambi
	cabbage, various	pl film				1w -ref
	carrot	pl film				1w -ambi
	carrots	pl bag				1w -ref
	celery	pl bag				1w -ambi
	endive	pl film				1w -ref
	haricots verts	pl box				1w -ambi
	icicles	pl bag				1w -ambi
	lettuce	pl film				1w -ref
	maize-ear	pl film				1w -ambi
	mushrooms	pl box				1w -ref
	onions	pl net				1w -ambi
	paprikas	pl film				1w -ambi
	potatoes	pl bag				1w -ambi
	red peppers	pl bag				1w -ambi
	tomatoes	pl box				1w -ambi
04.05	processed vegetables:					
	A. dried of dehydrated vegetables, whole or in the form of flour of powder					
	cabbage, various	alufoil				<1y -amb
	B. vegetables, cut or in the form of purée					
	potato, cut	pl bag	X			1d -ref
	rhubarb, cut	pl bag		X		1w ref
	selected vegetables for bami	pl tray/pl film	X			1w -ref
	tomato-puree	coated board		X		>1y-ambi
	tomato-puree	can		X		0.5h -st
	instant potatoes	pl bag				1w -dfr

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
	C. preserved vegetables:					
	sauerkraut	pl bag		X		3m -ambi
	I. in an aqueous medium					
	beans, various	glass pot/twist-off cap	X			1h -ster
	celery salade	glass pot/twist-off cap	X			1h -past
	cocktail mix	glass pot/twist-off cap	X			1h -past
	gherkin	glass pot/twist-off cap		X		1h -past
	green peas	glass pot/twist-off cap	X			1h -ster
	marrowfat pea	can	X			1h -ster
	olives, filled	glass pot/twist-off cap	X			0.5h -pas
	piccalilli	glass pot/twist-off cap		X		0.5h -pas
	red cabbage	glass pot/twist-off cap	X			1h -ster
	silver onions	glass pot/twist-off cap		X		0.5h -pas
	vegetables in pickle	glass pot/twist-off cap		X		0.5h -pas
	vegetables preserves, various	can	X(a)	X(a)		1h -ster
	vegetables salad	glass pot/twist-off cap	X			0.5h -pas
	wine-sauerkraut	glass pot/twist-off cap		X		0.5h -pas
	II. in an oily medium					
	III. in an alcoholic medium (≥ 5% vol.)					
05.	**fats and oils**					
05.01	animal and vegetable fat, whether natural or treated (including cocoa butter, lard, resolidified butter)					
	cooking fat	paper			X	1y -ambi
	corn oil	pl bottle/pl closure			X	>1y-ambi
	frying fat	paper			X	6m -ambi
	sunflower oil	pl bottle/pl closure			X	>1y-ambi
05.02	margarine, butter and other fats and oils made from water-in-oil emulsions					
	butter	paper/al lam			X/2	3m -ref
	halvarine	pl tub			X/2	3m -ref
	margarine	paper			X/2	3m -ref
06.	**animal products and eggs**					
06.01	fish:					
	A. fresh, chilled, salted, smoked					
	eel	pl sachet	X		X/3	2w -ref
	mackerel, smoked	pl sachet	X		X/3	3m -ambi
	salmon	pl vacupack	X		X/3	2w -ambi
	B. in the form of paste					
06.02	crustaceans and molluscs (including oysters, mussels, snails) not naturally protected by their shells					

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur	
06.03	meat of all zoological species (including poultry and game):						
	A. fresh, chilled, salted, smoked						
	beef heart	pl tray/pl film	X			X/4	1w -ref
	beefsteak	pl tray/pl film	X			X/4	1w -ref
	beef, slices	pl tray/pl film	X			X/4	1w -ref
	chicken chops	pl tray/pl film	X			X/4	1w -ref
	chicken filet	pl tray/pl film	X			X/4	1w -ref
	chicken, pieces	pl tray/pl film	X			X/4	1w -ref
	hamburger	pl tray/pl film	X			X/4	1w -ref
	liver	pl tray/pl film	X			X/4	1w -ref
	pork chop	pl tray/pl film	X			X/4	1w -ref
	rib of beef	pl tray/pl film	X			X/4	1w -ref
	steak	pl tray/pl film	X			X/4	1w -ref
	turkey schnitzel	pl tray/pl film	X			X/4	1w -ref
	turkey, pieces	pl tray/pl film	X			X/4	1w -ref
	turkey, sausages	pl tray/pl film	X			X/4	1w -ref
	B. in the form of paste or cream						
	beef rolls	pl tray/pl film	X			X/4	1w -ref
	beef, minced	pl tray/pl film	X			X/4	1w -ref
	brawn	pl tray		X		X/4	1m -ref
	liver pie	can	X			X/4	0.5h -st
	minced meat, mixed	pl tray/pl film	X			X/4	1w -ref
	pate ardennois	pl tray	X			X/4	1w -ref
	pate spread	pl tray	X			X/4	1m -ref
	pork rolls	pl tray/pl film	X			X/4	1w -ref
06.04	processed meat products (ham, salami, bacon and other)						
	bacon, sliced	pl sachet	X			X/4	1w -ref
	cooked sausage, sliced	pl sachet	X			X/4	1w -ref
	corned beef	can	X			X/4	0.5h -st
	corned beef, sliced	pl sachet	X			X/4	1w -ref
	ham	can	X			X/4	1h -ster
	ham, sliced	pl sachet	X			X/4	1w -ref
	liver sausage, sliced	pl sachet	X			X/4	1w -ref
	liver sausage	casing	X			X/4	1h -cook
	luncheon meat	can	X			X/4	0.5h -st
	minced-meat ball	pl vacupack	X			X/4	1m -ref
	salami	casing	X			X/4	1w -ambi

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	 C	 D	contact condition (time-temperatur	
	sausage	pl bag	X			X/4	3m -ambi
	sausages, various	pl sachet	X			X/4	1y -ambi
	sausage, sliced	pl sachet	X			X/4	3m -ref
	saveloy, sliced	pl sachet	X			X/4	3m -ref
	smoked sausage	pl bag	X			X/4	1m -ambi
							1m -ambi
06.05	preserved and partly preserved meat and fish:						
	A. in an aqueous med ium						
	bismarck herring	glass pot/twist-off cap		X			0.5h past
	herring filets in tomato sauce	can		X			0.5h -st
	crab	can	X				0.5h -st
	minced-meat balls	can	X				0.5h -st
	minced-meat soup balls	can	X				0.5h -st
	mussels	can	X				0.5h -st
	mussels in pickle	glass pot/twist-off cap		X			0.5h -pas
	Frankfurter	can	X				0.5h -st
	sausages	glass pot/twist-off cap	X				0.5h -st
	sausages	can	X				0.5h -st
	shrimps	can	X				0.5h -st
	B. in an oily me dium						
	anchovy	can				X	0.5h -st
	fish in oil, various	can				X	0.5h -st
	salmon	can				X	0.5h -st
	sardines	can				X	0.5h -st
	tuna fish	can				X	0.5h -st
06.06	eggs not in shell:						
	A. powdered or dried						
	eggs, whole, powdered	can					>1y -amb
	B. other						
06.07	egg yolk						
	A. in liquid form						
	B. frozen or powdered						
06.08	dried white of egg						
07.	**dairy products**						
07.01	milk:						
	A. whole milk						
	cocoa milk	pl bottle/pl closure	X				6m -ambi
	cocoa milk	coated board	X				6m -ambi
	milk	coated board	X				1w -ref
	milk	coated board	X				6m -ambi
	milk	pl bottle/pl closure	X				6m -ambi

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
	B. partly dehydrated					
	coffee creamer	coated board	X			6m -ambi
	coffee creamer	al cup	X			6m -ambi
	milk, condensed	can	X			0.5h -st
	C. skimmed or partly skimmed					
	curdled milk	pl tub	X			1w -ref
	D. dried					
07.02	fermented milk products like yoghurt and buttermilk and mixtures thereof with fruit or fruit products					
	buttermilk	coated board		X		1w -ref
	dressing (yoghurt), fresh	pl pot		X		3m -ref
	yoghurt	coated board		X		1w -ref
	yoghurt	pl pot		X		3m -ref
	yoghurt drink	coated board		X		6m -ambi
07.03	cream and sour cream					
	cream	pl pot	X			1w -ref
07.04	cheese:					
	A. whole and with rind					
	cheese, various a	rind				1w -ref
	B. processed					
	cheese, various	pl tub/pl film	X		X/3	1w -ref
	C. other					
	brie soft cheese	pl film	X		X/3	2w -ref
	cheese spread	al cup	X		X/3	1y -ref
	cheese, grinded	pl sachet	X		X/3	1m -ref
	cheese, sliced	pl box	X		X/3	3m -ref
	cheese, various	pl film/p tub	X		X/3	3m -ref
	cottage cheese	pl pot	X			1w -ref
	feta	pl tray		X		3m -ref
	monchou soft cheese	paper/al lam	X		X/3	3m -ref
	paturain soft cheese	pl tub/pl closure	X		X/3	1m -ref
	stmoret soft cheese	pl tray/pl closure	X		X/3	3m -ref
07.05	rennet:					
	A. in liquid or semi-liquid form					
	B. powdered or dried					
07.06	milk products, processed					
	custard, various	coated board	X			6m -ambi
	custards, various	coated board	X			1w -ref
	pudding	pl pot	X			3m -ref

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
08.	**miscellaneous products**					
08.01	vinegar					
	vinegar	pl bottle/pl closure		X		>1y-ambi
08.02	fried or roasted foods:					
	A. fried potatoes, fritters and the like					
	chips, various	pl bag			X/5	6m -ambi
	instant patates frites	pl bag			X/5	>1y-dfr
	kroepoek	pl bag			X/5	3m -ambi
	B. of animal origin					
	beefburger	alufoil pack			X/4	1y -ambi
	hamburger	alufoil pack			X/4	1y -ambi
	hot dog, prep	coated board			X/4	1y -dfr
08.03	preparations for soups or broth, in liquid or powder form, extracts and concentrates, prepared dishes, homogenised composite food preparations:					
	A. powdered or dried					
	I. with fatty substance on the surface					
	dishes, various	pl pot			X/5	3m -ambi
	meat juice, powdered	pl sachet			X/5	>1y-ambi
	sauce powder, various	pl sachet			X/5	1y -ambi
	soup powder, various	alufilm			X/5	>1y-ambi
	II. other					

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
	B. liquid or paste:					
	I. with fatty substance on the surface					
	babi pangang, prep	alutray	X			X/3 1y -dfr ·
						1y -dfr ·
	babi sateh, prep	alutray	X			X/3 1y -dfr ·
						1y -dfr ·
	baked rice, prep	coated board	X			X/3 2w -ref
	beef salade	pl tray	X	X		X/3 1m -ref
	cannelloni, prep	coated board	X			X/3 1y -dfr ·
						1y -dfr ·
	chicken curry, prep	coated board	X			X/3 1y -dfr ·
						1y -dfr ·
	lasagna, prep	alu tray	X			X/3 1y -dfr ·
						1y -dfr ·
	macaroni dish	can	X			X/3 0.5h -st
	meat extract	glass pot/twist-off cap	X			X/3 0.5h -st
	mihoun with chicken, prep	alu tray	X			X/3 1y -dfr ·
						1y -dfr ·
	paella, prep	coated board	X			X/3 1y -dfr ·
						1y -dfr ·
	quiche, prep	coated board	X			X/3 2w -cool
	ragout	can	X			X/3 0.5h -st
	salads, various	PL tray		X		X/3 1w -reef
	salmon with herbs, prep	coated board	X			X/3 1w -reef
	spaghetti, prep	salutary/alveoli	X			X/3 1y -ambi
	vegetarian hamburger	PL tray/PL film	X			X/3 1w -reef
	vegetarian kebab	PL tray/PL film	X			X/3 1w -reef
	vegetarian schnitzel	PL tray/PL film	X			X/3 1w -reef
	II. other					
	potato/endive, prep	salutary/alveoli	X			1w -ambi
	tomato soup, prep	PL pot/PL closure		X		2w -reef
08.04	yeast and raising agents:					
	A. in the form of paste					
	B. dried					
08.05	table salt					

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	C	D	contact condition (time-temperatur
08.06	sauces:					
	A. no fatty substance on the surface					
	ketchup	PL bottle/PL closure		X		1y -ambi
	ketchup	glass bottle/pl closure		X		1y -ambi
	salade dressing, light	glass bottle/pl closure	X	X		>1y-ambi
	sauces, various	glass bottle/pl closure		X		6m -ambi
	soy-bean sauce	pl bottle/pl closure	X			1y -ambi
	B. mayonnaise, sauces derived from mayonnaise, salad dressings and other oil-in-water emulsions					
	fried potato sauce	pl pot/pl closure		X	X/3	6m -ambi
	mayonnaise	glass pot/twist-off cap		X	X/3	>1y-ambi
	mayonnaise	al tube/pl closure		X	X/3	6m -ambi
	salade cream	glass bottle/pl closure		X	X/3	6m -ambi
	salade dressing	glass bottle/pl closure		X	X/3	6m -ambi
	sandwich spread	glass pot/twist-off cap		X	X/3	0.5h -pas
	sauces, various	glass pot/twist-off cap	X(a)	X(a)	X/3	0.5h -pas
	sauces, various	glass bottle/pl closure	X(a)	X(a)	X/3	(0.5h pas
	C. containing oil and water in distinct layers					
08.07	mustard (except powdered mustard as under heading 08.17)					
	mustard	glass pot/pl closure		X	X/3	>1y-ambi
08.08	sandwiches, toasted bread and the like containing any kind of foodstuff:					
	A. with fatty substance on the surface					
	sandwiches	pl bag			X/5	1d -ambi
	B. other					
08.09	ice cream					
	ice-cream	pl box	X			>1y-dfr1
	ice-cream, magnum	paper				1y -dfr
	ice-cream, snickers	paper				1y -dfr
	ice-lolly	paper				>1y-dfr
08.10	dried foods:					
	A. with fatty substance on the surface					
	B. other					

ref. no.	description of foodstuffs	food contact material A	simulants to be used B	 C	 D	contact condition (time-temperatur
08.11	frozen or deep-frozen foods					
	bavarois pudding	coated board				1y -dfr
	beans	coated board				1y -dfr
	cod filet	coated board				1y -dfr
	croquettes	coated board				1y -dfr
	cuttle-fish	coated board				1y -dfr
	green cod	coated board				1y -dfr
	green peas	coated board				1y -dfr
	hamburgers	coated board				1y -dfr
	herring	coated board				1y -dfr
	loempias	coated board				1y -dfr
	minced-meat balls	coated board				1y -dfr
	minced-meat balls	pl box				1y -dfr
	minced-meatb balls	pl bag				1y -dfr
	pastries, various	coated board				3m -dfr
	pizza	coated board				>1y-dfr
	potato croquettes	pl bag				>1y-dfr
	prepared dishes, various	coated board				1y -dfr
	rolled chicken	coated board				1y -dfr
	shrimps	coated board				1y -dfr
	spiced loempias	coated board				1y -dfr
	vegetable dish	coated board				1y -dfr
	vegetables, cut	coated board				1y -dfr
08.12	concentrated alcoholic extracts of an alcoholic strength equal to or exceeding 5% vol.					
08.13	cocoa:					
	A. powder					
	B. paste					
	cocoa paste	pl pot/pl closure			X/3	1m -ambi
08.14	coffee, whether or not roasted, decaffeinated of solubilized, coffee substitutes, granulated or powdered					
	coffee, powdered	alufoil				1m -ambi
	coffee, powdered	can				>1y-ambi
08.15	liquid coffee extract					
08.16	aromatic herbs and other herbs (camomile, mallow, mint, tea, lime blossom, etc.):					
08.17	spices and seasonings in the natural state (cinnamon, cloves, powdered mustard, pepper, vanilla, saffron, etc.):					
	spices, various	paper/al sachet				>1y-ambi
	spices, various	pl sachet				>1y-ambi

REFERENCES

1) According to Directive 93/8/EEC only a test at 40°C for 10 days is required.

2) This test shall be carried out only in cases where the pH is 4.5 or less.

3) This test may be carried out in the case of liquids or beverages of an alcoholic strength exceeding 15% vol. with aqueous solutions of ethanol of a similar strength.

4) Test to be carried out with iso-octane

5) or 2 h - 70°C and 10 days at 40°C separately, according to Directive 93/8 EEC.

6) If it can be demonstrated by means of an appropriate test that there is no 'fatty contact' with the plastic, the test with simulant D may be dispended with

7) Simulant A: 10 d - 40°C + 0.5 h - 100°C

8) Simulant D may be replaced with iso-octane using test conditions of 1 d - 20°C

9) Simulant D may bereplaced with iso-octane using test conditions of 1 h - 60°C

10) Simulant D may be replaced with iso-octane using test conditions of 1 d - 5°C

THE END