STABILITY OF GLUCOSINOLATES IN SEEDS AND IN RAPESEED EXTRACTS UNDER DIFFERENT PACKAGING CONDITIONS

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INTRODUCTION

Rapeseed is virtually the only oil seed which can be grown economically in the northern latitudes. It is cultivated mainly as a rich source of valuable oil. The cake which remains after the oil is expelled from the seed is widely used as a feed for farm animals and poultry. Unfortunately, the use of rapeseed cake is limited by the presence of a number of antinutritional factors the most of which are glucosinolates (GLS).

Introduction by the European Community (EEC) of a premium payment to producers of low GLS rapeseed has brought the need for an accurate, official method of analysis and for reference materials (RMs). It is in the context of the need for RMs that the Community Bureau of Reference (BCR) has undertaken a collaboration project to evaluate stability of intacts glucosinolates in seeds and of desulphoglucosinolates extracts.

MATERIALS AND METHODS

Seeds used for the present study are of French origin (Darmor variety).

The method used to determine GLS in seeds is HPLC of desulphoglucosinolates which is now the official method of the EEC (EEC Journal n° L170/28 03/07/90)

The desulphoglucosinolate extract is prepared as follow: GLS are extracted from seeds with hot methanol (70/30),purified with an amberlite column(CG 120 H+ 28-35 mesh). GLS changed in desulphoglucosinolates by action of a sulphatase (Helix pomatia) are eluated on a DEAE sephadex A 25 column.

RESULTS

STABILITY OF GLUCOSINOLATES IN SEEDS

Before preparing reference materials it is important to study the stability of the GLS content of the seeds under different packaging conditions. Seeds (20 g) were stored in heat sealed laminated bags at four temperatures (-18°c, 4-6°c, 20°c and 28°c) under vacuum, air and nitrogen.

a) Total GLS content evolution

Influence of temperature

Table 1 shows that temperature is an important factor. For

each packaging condition and after 30 months, the total GLS content decreases slowly with increase in temperature.

Table 1. Influence of storage time on total GLS content (µmol/g) according to temperature and packaging conditions

		time of storage in months						
	T°	0	3	6	12	18	24	30
vacuum	-18°c	24.10	25.17	25.30	25.22	25.21	25.13	25.05
	4-6°c	24.40	24.73	24.98	24.92	24.99	24.88	24.82
	20°c	24.35	24.70	25.02	24.87	24.91	24.77	24.72
	28°c	25.35	24.57	24.90	24.84	24.30	24.60	24.41
	Mean	24.55	24.79	25.05	24.96	24.98	24.85	24.75
air	-18°c	24.75	24.88	25.06	25.08	24.97	24.93	24.86
	4-6°c	24.75	24.31	24.62	24.57	24.63	24.56	24.54
	20°c	25.36	24.56	24.90	24.80	24.85	24.78	24.65
	28°c	23.50	24.39	24.72	24.62	24.54	24.34	24.20
	Mean	24.58	24.54	24.82	24.77	24.75	24.65	24.56
nitrogen	-18°c	24.25	24.22	24.71	24.75	24.92	24.91	24.85
	4-6°c	24.65	24.45	24.85	24.80	24.80	24.71	24.66
	20°c	22.45	24.30	24.32	24.27	24.35	24.24	24.21
	28°c	25.25	23.80	24.11	24.18	24.14	23.91	23.79
	Mean	24.15	24.20	24.50	24.50	24.55	24.44	24.38
TOTAL MEAN		24.42	24.51	24.79	24.74	24.76	24.65	24.56

Influence of packaging conditions

After 30 months, the mean of all results obtained for seeds stored under vacuum (24.75 µmol/g) is higher than for seeds stored under air (24.56 µmol/g) and nitrogen (24.38 µmol/g).

Influence of the storage time

It is not possible to observe a systematic variation of the total glucosinolate content. Any differences are probably due to the repeatability of the HPLC method.

Interaction between temperature and packaging

If we compare the three different type of package,there are no significant differences for the seeds stored at -18°c or for seeds stored under vacuum at -18°c, 4-6°c or 20°c.

b) Alkenyl and indolyl content evolution

The influence of temperature , packaging and time of storage on the alkenyl glucosinolates is very similar to total GLS content. For the alkenyl GLS, we have not observed significant differences for seeds stored at -18°c .

Concerning the indolyl GLS, the temperature and the storage time have some influence but the type of packaging does not influence the results.

STABILITY OF DESULPHOGLUCOSINOLATES EXTRACTS

To control the calibration of the HPLC equipment it is usefull to have a reference material with a known content of each individual GLS. According to the official method, the desulphoglucosinolates are separated by HPLC. It is therefore necessary to know the stability of extracts of these compounds.

Desulphoglucosinolates extracts were sealed in glass ampoules in a liquid or a solid form after lyophilisation, under vacuum or nitrogen with and without antioxydants at three temperatures (-18°c, 4°c and 20°c). Liquid solutions are in water, buffer 1 (acetic acid 0.02M + NaOH 2N to pH 3.74) and buffer 2 (acetic acid 0.02M + NaOH 2N to pH 5). Antioxydants are a mixture of BHT, BHA, octylgallate and ascorbylpalmitate (20 mg/100 ml).

During 192 days stability was studed for all the storage conditions .

Table 3. Composition of the extracts after 192 days of storage (ratio with sinigrin)

	vacuum	+ANT	nitrogen	+ANT	water	+ANT	buffer 1	+ANT	buffer 2	+ANT
PROGOITRIN										
-18°c	8.94	8.84	8.84	8.80	8.79	8.87	8.81	8.89	8.97	8.87
4°c	8.84	9.02	8.78	8.92	8.89	8.91	8.89	8.89	8.96	8.86
20°c	10.20	8.92	9.11	10.40	8.64	8.98	8.92	9.47	8.84	8.82
NAPOLEIFERIN										
-18°c	0.39	0.45	0.45	0.45	0.44	0.43	0.47	0.43	0.46	0.44
4°c	0.43	0.55	0.44	0.43	0.44	0.48	0.43	0.43	0.47	0.44
20°c	0.47	0.45	0.43	0.48	0.42	0.55	0.37	0.63	0.46	0.46
GLUCONAPIN										
-18°c	2.60	2.59	2.55	2.54	2.58	2.62	2.55	2.58	2.61	2.61
4°c	2.59	2.81	2.58	2.58	2.63	2.63	2.60	2.58	2.65	2.62
20°c	2.71	2.59	2.61	2.85	2.53	2.61	2.63	2.70	2.57	2.57
4 OH GLUCOBRASSICIN										
-18°c	1.33	1.34	1.34	1.27	1.00	1.15	1.00	1.34	1.18	1.47
4°c	1.19	1.17	1.06	0.60	1.09	1.02	1.14	1.11	1.15	1.19
20°c	0.33	1.32	0.98	0.35	1.03	0.89	0.23	0.96	0.89	1.07
GLUCOBRASSICANAPIN										
-18°c	0.98	0.94	0.96	0.94	0.94	0.96	0.98	0.95	0.98	0.97
4°c	0.98	1.01	0.97	0.94	0.98	0.96	0.98	0.95	0.99	0.97
20°c	1.00	1.00	1.03	1.11	0.92	0.96	0.96	1.00	0.98	0.93
GLUCOBRASSICIN										
-18°c	0.15	0.16	0.18	0.15	0.15	0.16	0.15	0.17	0.18	0.17
4°c	0.16	0.15	0.15	0.13	0.15	0.15	0.15	0.15	0.15	0.17
20°c	0.15	0.16	0.15	0.16	0.16	0.21	0.15	0.17	0.16	0.16
NEOGLUCOBRASSICIN										
-18°c	0.25	0.36	0.36	0.51	0.26	0.26	0.26	0.64	0.51	0.47
4°c	0.27	0.25	0.26	0.47	0.34	0.30	0.40	0.27	0.42	0.35
20°c	0.19	0.45	0.34	0.45	0.29	0.69	0.20	0.32	0.39	0.32

Table 2. Influence of storage time on alkenyl and indolyl GLS content (μmol/g) according to temperature and packaging conditions

a) alkenyl glucosinolates

	T°	time of storage in months 0	3	6	12	18	24	30
vacuum	-18°c	20.20	21.07	21.04	20.95	20.93	20.90	20.84
	4-6°c	20.15	20.64	20.77	20.70	20.72	20.67	20.63
	20°c	20.10	21.00	21.01	20.86	20.85	20.77	20.75
	28°c	21.10	20.60	20.80	20.75	20.75	20.68	20.57
	Mean	20.39	20.83	20.91	20.81	20.81	20.76	20.70
air	-18°c	20.55	20.59	20.91	20.94	20.52	20.81	20.75
	4-6°c	20.90	20.33	20.48	20.45	20.47	20.45	20.44
	20°c	21.35	20.78	20.53	20.72	20.73	20.70	20.62
	28°c	19.70	20.57	20.72	20.64	20.62	20.55	20.48
	Mean	20.63	20.64	20.74	20.69	20.66	20.63	20.57
nitrogen	-18°c	20.30	20.17	20.49	20.52	20.65	20.68	20.64
	4-6°c	20.50	20.44	20.68	20.63	20.62	20.57	20.53
	20°c	18.60	20.25	20.15	20.12	20.20	20.17	20.18
	28°c	20.90	19.59	20.10	20.17	20.18	20.09	20.05
	Mean	20.07	20.19	20.35	20.36	20.41	20.38	20.35
TOTAL MEAN		20.36	20.55	20.67	20.62	20.63	20.59	20.54

b) indolyl glucosinolates

	T°	time of storage in months 0	3	6	12	18	24	30
vacuum	-18°c	3.90	4.01	4.28	4.29	4.29	4.24	4.22
	4-6°c	4.25	4.09	4.21	4.22	4.27	4.21	4.19
	20°c	4.25	3.98	4.20	4.17	4.19	4.12	4.08
	28°c	4.25	3.83	4.10	4.09	4.05	3.80	3.84
	Mean	4.16	4.04	4.19	4.19	4.20	4.12	4.08
air	-18°c	4.25	4.01	4.15	4.15	4.17	4.12	4.11
	4-6°c	3.90	4.05	4.17	4.15	4.19	4.13	4.12
	20°c	4.00	4.09	4.27	4.26	4.27	4.20	4.14
	28°c	3.85	3.83	3.99	3.97	3.92	3.80	3.72
	Mean	4.00	3.99	4.15	4.13	4.14	4.06	4.02
nitrogen	-18°c	3.95	4.05	4.23	4.23	4.27	4.22	4.21
	4-6°c	4.00	4.03	4.18	4.17	4.19	4.15	4.14
	20°c	3.90	4.06	4.18	4.16	4.16	4.08	4.04
	28°c	4.35	3.95	4.03	4.03	3.98	3.84	3.76
	Mean	4.05	4.03	4.16	4.15	4.15	4.07	4.04
TOTAL MEAN		4.07	4.02	4.17	4.16	4.16	4.09	4.05

After 192 days of storage, progoitrin, gluconapin, glucobrassicanapin, napoleiferin, glucobrassicin and neoglucobrassicin seem to be stable under vacuum, nitrogen, in water, in buffered solutions with and without antioxydants at the three temperatures considered. At minus 18°c, 4 OH glucobrassicin also appears to be stable under vacuum and nitrogen but degrades in the other storage conditions, especially in liquid solutions. At 4°c and 20°c we observed in all storage conditions, a loss of 4 OH glucobrassicin.

After this first study it was decided to continue the stability study only with vacuum and nitrogen ampoules stored at -18°c.

The research is not yet finished. Table 4. shows the results obtained at -18°c during 760 days.

Table 4. Stability of desulphoglucosinolates extracts at -18°c (ratio with sinigrin)

	Days	1	29	100	192	300	425	549	760
PRO	VACUUM	9.00	9.24	9.16	8.94	8.99	9.07	9.22	8.91
	VAC.+ANT.	8.88	9.21	9.00	8.84	8.78	9.10	9.07	8.85
	NITROGEN	8.97	9.25	8.89	8.84	8.76	9.20	8.99	8.93
	NIT.+ANT.	8.98	9.21	8.74	8.81	8.67	9.12	8.87	8.78
EPRO	VACUUM	0.20	0.37	0.17	0.16	0.19	0.18	0.16	0.14
	VAC.+ANT.	0.22	0.29	0.26	0.20	0.00	0.37	0.15	0.15
	NITROGEN	0.20	0.21	0.35	0.20	0.00	0.33	0.14	0.14
	NIT.+ANT.	0.27	0.26	0.23	0.19	0.00	0.39	0.15	0.14
GNL	VACUUM	0.40	0.44	0.42	0.39	0.44	0.46	0.39	0.31
	VAC.+ANT.	0.39	0.41	0.41	0.45	0.43	0.46	0.40	0.29
	NITROGEN	0.41	0.41	0.58	0.45	0.44	0.40	0.39	0.29
	NIT.+ANT.	0.40	0.41	0.37	0.45	0.45	0.45	0.38	0.30
GNA	VACUUM	2.62	2.65	2.86	2.60	2.61	3.00	2.66	2.61
	VAC.+ANT.	2.58	2.62	2.96	2.59	2.56	2.64	2.63	2.61
	NITROGEN	2.62	2.65	2.57	2.55	2.63	2.61	2.64	2.61
	NIT.+ANT.	2.66	2.62	2.61	2.54	2.57	2.58	2.62	2.60
4OH	VACUUM	1.53	1.33	1.43	1.33	1.27	1.11	1.66	1.21
	VAC.+ANT.	1.39	1.34	1.28	1.34	1.63	1.16	1.65	1.31
	NITROGEN	1.50	1.22	1.34	1.34	1.69	1.08	1.62	1.17
	NIT.+ANT.	1.46	1.25	1.48	1.27	1.52	1.02	1.47	1.19
GBN	VACUUM	0.98	1.08	1.02	0.98	0.97	1.24	1.06	0.94
	VAC.+ANT.	0.96	1.07	1.00	0.94	0.97	1.24	1.04	0.81
	NITROGEN	0.99	1.02	0.93	0.96	1.22	0.97	1.06	0.99
	NIT.+ANT.	0.98	1.03	1.06	0.94	0.94	0.97	1.05	0.98
GBC	VACUUM	0.18	0.17	0.22	0.15	0.15	0.19	0.15	0.14
	VAC.+ANT.	0.18	0.17	0.19	0.16	0.15	0.39	0.13	0.13
	NITROGEN	0.21	0.23	0.25	0.18	0.19	0.14	0.14	0.13
	NIT.+ANT.	0.19	0.15	0.31	0.15	0.15	0.14	0.14	0.13
NAS	VACUUM	0.41	0.46	0.42	0.36	0.17	0.33	0.27	0.28
	VAC.+ANT.	0.28	0.23	0.29	0.36	0.30	0.45	0.28	0.28
	NITROGEN	0.23	0.43	0.41	0.40	0.22	0.48	0.23	0.28
	NIT.+ANT.	0.22	0.30	0.27	0.49	0.22	0.38	0.27	0.28
NEO	VACUUM	0.32	0.42	0.30	0.25	0.26	0.28	0.25	0.24
	VAC.+ANT.	0.35	0.31	0.28	0.36	0.32	0.34	0.24	0.25
	NITROGEN	0.39	0.28	0.31	0.36	0.26	0.25	0.24	0.26
	NIT.+ANT.	0.34	0.27	0.58	0.51	0.27	0.29	0.23	0.25

After 760 days of storage at minus 18°c it appears that

vacuum ampoules offer the best storage conditions. The differencies in OH glucobrassicin content may be due to method effects althought the same HPLC column and operating conditions were used.However, we believe that it is necessary to continue the study and to control especially the evolution of 4 OH glucobrassicin.

CONCLUSIONS

Stability of intact glucosinolates in seeds is improved with sachets under vacuum or under nitrogen. The storage temperature will be -18°c or eventually 4-6°c.

Concerning the stability of desulphoglucosinolate extracts, it appears that the 4 OH glucobrassicin is less stable especially at 4°c and 20°c. The stability study is not finished but we believe, at the present time,that the best storage condition is under vacuum at minus 18°c.