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DEPROTEINIZATION INFLUENCE ON SPECTROPHOTOMETRIC DETERMINATION OF NITRATES AND NITRITES

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Abstract

Nitrites and nitrates are contaminants mostly for vegetal origin food through environmental factors or improper agricultural practices.

The main toxicological risk of nitrites is related to nitrosamine formation which was related to some cancer forms.

EU legislation sets Maximum Residues Level values for leafy vegetables and for baby food which are under sanitary-veterinary surveillance.

Several samples of lettuce, rocket salad, spinach and baby food were analysed in order to determinate their nitrites and nitrates content.

Griess spectrophotometric method using an aqueous and deproteinizated extracts of the samples was applied. Rocket salad samples show the highest nitrate content (2368 and 2177 mg/kg) on both of the tested extracts.

All leafy vegetal sampleshave a nitrate content below the legal specific requirement, only frozen spinach was close to MRL. So even if there are no significant differences between the results obtained using different extracts,thedeproteinization of the vegetal samples is mandatory for control purposes.

Key words: nitrates, nitrites, deproteinization, Griess method

INTRODUCTION

The foods are subject to contamination by chemical substances from different sources: raw material production, processing, packaging, handling, transportation and especially from environmental factors.

Regarding nitrites and nitrates, they contaminate mostly vegetal origin food due to inappropriate use of fertilisers or from polluted irrigation waters.

The storage conditions of vegetables influence their content of nitrites and nitrates (Chung et al., 2004).

Nitrites and nitrates are also admitted additives for processed meat products in order to preserve their colour and as antimicrobial agent.

Both optic and chromatographic methods are used in order to determinate those contaminants (Silalahi, et al, 2018, Khanfar et al, 2019, Scheeren et al, 2013**).**

Water or food contamination by nitrites can induce methemoglobinemia to babies, but the major risk of nitrites is related to the

formation of nitrosamines in the presence of secondary amines (Rostkowska, 1998).

Nitrosamines were found to be related to gastric and oesophageal cancer (Bambilla and Martelli, 2007, Jakszyn and González, 2006 a). For humans, the nitrates input are exogenous, but for nitrites it is preponderant endogenous due to the conversion of nitrates to nitrites in the gastric acidic environment (EFSA Journal, 2008).

Therefore, even if the toxic effect of nitrites is higher than the nitrates one, both European (CE 1881/2006) and national legislation (ANSVSA Order nr 97/2007) stipulates Maximum Residues Levels (MRL) for nitrates in order to avoid the accumulation of nitrites through a reduction process.

The MRL values refer tocertain leafy vegetables such as spinach, lettuce and rocket salad depending on the cultivation area and period, as well as for baby food.

The presence of nitrates in food is subject to continuous monitoring and Romania is an important contributor to this issue, 20% of total analysed data was from Romania, second after Germany with 34% (EFSA, 2008).

The results from 2008 that refers to the precedent seven years pointed to rocket salad as the most affected vegetable by nitrates contamination.

So, the European legislation was amended by Regulationnr. 1258/2011 andthis leafy vegetablewas added among those under sanitary veterinary surveillance.

National studies about the intake of nitrites and nitrates through foods were conducted in Spain (Jakszyn et al, 2006 b), Estonia (Tamme et al, 2006) and Portugal (Rebelo et al., 2015).

The aim of this study was to verify the influence of the deproteinizationof some vegetables samples on the nitrites and nitrates determination by a spectrophotometric method.

MATERIAL AND METHOD

Material

The tested materials consist in samples of vegetal origin chosen from the one listed with MRL valuesas follows:Fresh spinach (*Spinaciaoleracea*) (FSP) and preserved, frozen spinach (PSP); Fresh Lettuce (*Lactucasativa*)– green house cultivated during winter (FLGW) and summer (FLGS); Fresh Lettuce (*Lactucasativa*) – field cultivated during winter (FLFW) and summer (FLFS); Iceberg Lettuce – green house (ILG) and fieldcultivated (ILF); Rocket salad (*Erucasativa*) – cultivated during winter (RW) and summer (RS); Baby food in jar, BF1 containspotatoes and spinach and BF2 containsrice, vegetables and spinach.

The above-mentioned codes are used all along this paper. The samples were purchased from local shops in Oradea during September 2018 – July 2019 and the experiments were conducted in the food control laboratory of the Food Engineering Department, Environmental Protection faculty.

Methods

The determination of nitrites was performed using the Griess spectrophotometric method (Hura, 2006). Freshly dried natrium nitrite was the standard for0.005 and 0.01 mg nitrite.

The nitrates concentration was determined indirectly after the reduction of nitrates to nitrites with cadmium and the redetermination of nitrites in the reduced extract.

Two experimental variants were applied:

1- Aqueous extracts: 10 g of homogenised sample were mixed in a 250 ml flask with 100 ml hot water andkept for 30 minutes at 80°C. Then, water was added to the mark and after cooling, a clear extract was obtained by centrifugation 5 minutes at 5000 rot/min.

2 - Deproteinizated extracts: to the same amount of sample and water as for (1) variant 5 ml saturated borax solution was added and heated for 15 minutesat80°C, stirring it from time to time. After cooling at room temperature 6ml of 10.6% potassium ferrocyanure solution and 6 ml 22% zinc acetate solution ware added.After 20-30 minutes the clear extract was again obtained by centrifugation.

From the filtrate obtained through the two above mentioned procedures, 10 ml were added to 10 ml ofGriess reagent, stirred and let to rest for at least 20 min, but no more than 4 hours, in the dark, at room temperature. The absorbance was measured with a Shimadsu Mini UV-VIS spectrophotometer at 520nm wavelength towards a blank solution of the reagents. Two determinations were performed for each sample or standard.

RESULTS AND DISCUSSION

The first step of the experiment was to obtain the foods aqueous and the deproteinizated extracts, then nitrites and nitrates were calculated for both series of extracts as explain above.

The results, as means +/- Sd are presented in Table 1.

The experimental results reveal for all the tested samples higher values using the deproteinizated extract than the aqueous one.

The differences between nitrites and nitrates content determined using the two different extracts are from 3.97% to 15.89% (nitrites) and from 3.76% to 17.46% (nitrates).

Statistical analysis (t-test) reveals not-significant differences; however, there is no predictability or relationship between the samples type and the extent of the differences.

Referring to the aqueous extracts of tested leafy vegetables, the lowest nitrites content (0.79 mg/kg) was found in FLGS sample and the highest (2.96 mg/kg) in FPS.

For nitrates, the lowest content (325.6 mg/kg) was found in FLGS sample and the highest (2177; 2018mg/kg) in rocket salad, regardless of the cultivation period. As for deproteinizated extracts, the lowest nitrites content (0.094 mg/kg) was found again in FLGS sample and the highest (3.375 mg/kg) in FPS.

Regarding nitrates, the lowest content (371.6 mg/kg) was found in FLGS sample and the highest (2368; 2097.2mg/kg) in rocket salad, regardless of the cultivation period.

Those results comply with the ones reported by scientific studies. Avasilcăi, 2012 found for indoor green salad an average content of 0.69 mg/kg of nitrite and 138.1 mg/kgnitrates.

Outdoor cultivated salad shown higher values, 2.24 mg/kg for nitrites and 1363.8 mg/kg for nitrates.Kmecl et al., 2017, sensitising a 13 years study in Slovenia, found values of 0.28 mg/kg nitrites and for nitrates 962 mg/kg lettuce.

In Estonia, a study from 2006 (Tamme et al, 2006) found an average under 5 mg/kg and for nitrites 2508 mg/kg for spinach.

Table1

Nitrites and nitrates content, mg/kg

Sample code		**NO2**	**NO3**	**NO2**	**NO3**
		Aqueous extracts		Deproteinizated extracts	
FSP	Mean	2.960	1076.16	3.375	1859.16
	Sd	+/-0.204	+/-87.99	+/-0.280	+/-24.61
PSP	Mean	1.456	1739.08	1.634	1817.10
	Sd	+/-0.218	+/-35.25	+/-0.147	+/-72.45
FLGW	Mean	1.894	470.97	2.138	550.11
	Sd	+/-0.400	+/-39.35	+/-0.564	+/-8.74
FLGS	Mean	0.791	325.58	0.094	371.62
	Sd	+/-0.136	+/-11.65	+/-0.022	+/-10.89
FLFW	Mean	1.684	1445.94	1.904	1617.03
	Sd	+/-0.390	+/-124.50	+/-0.397	+/-82.08
FLFS	Mean	1.294	897.16	1.411	1086.78

	Sd	+/-0.058	+/-37.67	+/-0.0803	+/-113.14
ILG	Mean	1.180	942.64	1.403	997.17
	Sd	+/-0.024	+/-78.72	+/-0.016	+/-122.08
ILS	Mean	1.466	928.60	1.649	1062.10
	Sd	+/-0.064	+/-57.19	+/-0.044	+/-20.59
RW	Mean	1.354	2177.04	1.411	2368.05
	Sd	+/-0.167	+/-145.31	+/-0.157	+/-158.98
RS	Mean	1.321	2018.37	1.403	2097.18
	Sd	+/-0.227	+/-131.08	+/-0.025	+/-38.58
BF1	Mean	0.339	36.82	0.413	38.58
	Sd	+/-0.013	+/-1.20	+/-0.014	+/-1.68
BF2	Mean	0.478	31.70	0.541	36.05
	Sd	+/-0.013	+/-2.27	+/-0.066	+/-1.52

We did not compare baby foods to the tested leafy vegetables because they are mixed foods and the MRL value is at least 10 times lower than for leafy vegetables, as we are going to discuss further on.

In Europe, the studies regarding this item found for nitrates values between 61 mg/kg in Portugal (Rebelo et al., 2015) and 88 mg/kg in Estonia (Tammi et al, 2006), so the samples analysed in the present study shown a lower contamination by nitrates.

The comparison of the tested samples with the MRL values of nitrates for each category of samples is presented in the graph from the Figure 1

.As we can see, all experimental values are beneath the maximum admitted, no matter the type of extract used in the experiment.

However the ones obtained from deproteinizated extract are always higher and in one case for preserved spinach it is very close to the MRL representing 91%.

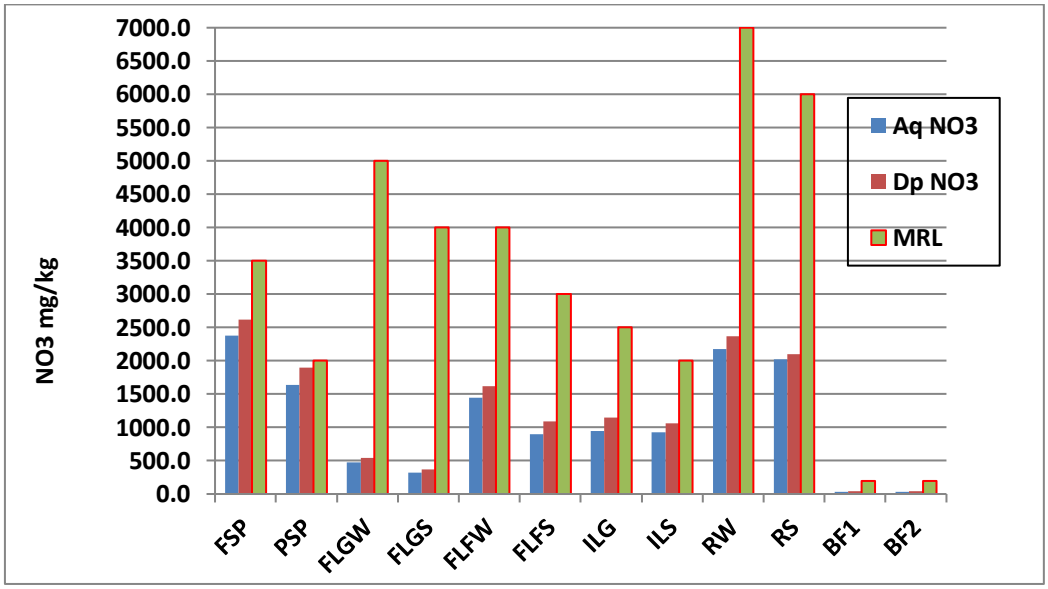

Fig.1 – The comparison of experimental nitrates content to MRL

CONCLUSION

The experimental results of the study reveal the fact that aqueous extracts can be used in order to determinate the nitrites and nitrates content of leafy vegetal origin food as well as for mixed vegetal origin baby foods in routine verifications. However if the registered values are near the MRL values, the deproteinizated extract should be used in order to avoid any discrepancywith the legal requirements. However, if the determination is related to a control activity, concluded with a control act, the deproteinization of the samples is mandatory.

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