Phytosterols and tocopherols variability in Linseed (Linum usitatissimum)

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INTRODUCTION

Linseed (*Linum usitatissimum L.*) is mainly cultivated in the North of France. Linseed oil is well known because of its high content on linolenic acid (ω3). This oil can be used both for human nutrition [1] and non-food uses but there is few information about the minor components [2, 3] present in the oil as well as their variability which may increase the interest on this oil.

The presence of high levels tocopherols and phytosterols in linseed oil could play an important role in the protection of the plant and the oil quality, specially in hot stress conditions.

The aim of this study was to evaluate the content and composition as well as the factors of variation (genotype, year and location) of these two families of minor components: tocopherols and phytosterols because of their nutritional interest or in non-food uses because of their antioxidant activity.

MATERIALS AND METHODS

Linseed samples

Samples were collected in two different places in the North of France (Airaines and Poix de Picardie) placed in the main linseed production area. The third location was in Lavaur in the South of France. In order to be able to cultivate in this place, irrigation was employed (Table 1).

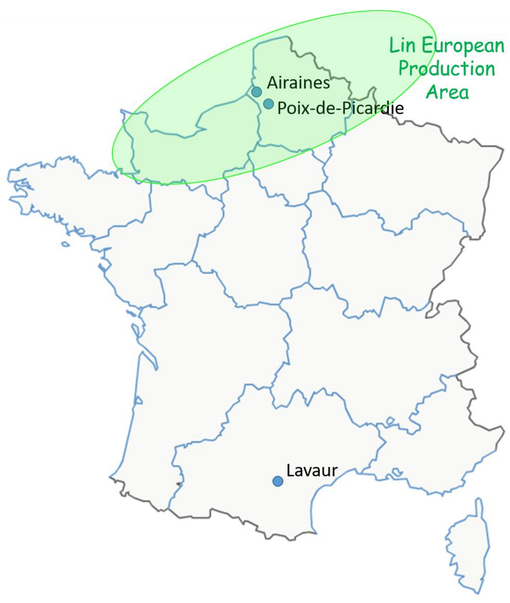

Table 1. Linseed sampling for each year

	YEAR		
	2013	2014	2015
Genotypes tested	6	10	6
Number of locations	2	3	1

Chemical analysis of seeds

LINSEEDS 10 seeds → Grinding → Oil extraction → Dilution Isooctane → HPLC-Fluorescence → TOCOPHEROLS QUANTIFICATION

Oil extraction → Saponification KOH (0.5%) → Purification Aluminium oxyde column → Silylation → GC-MS - FID → STEROLS QUANTIFICATION

RESULTS AND DISCUSSION

Linseed composition

Linseed oil presented 6 major phytosterols: Campésterol, Stigmasterol, β-sitosterol, avenasterol, cycloratenol and Δ7-Stigmasterol, where β-sitosterol was the most important with 36±3% of the total phytosterol content, followed by cycloartenol 22±3%.

Linseed also presented a high content of tocopherols in its oil, mainly γ and δ-tocopherol.

Effects of environment and genotype

Meteorological dataset shows that 2015 was the hottest and driest year both in French North and South weather conditions but not specially dry during the linseed growing period. The South conditions were always hottest than the Northern conditions whereas 2013 was the coldest. 2014 was the rainiest year. These significant differences in the weather during the growing period of linseed were probably responsible for the modifications in the content and composition of minor components, independently of genotype. Further analysis on the impact of weather conditions during grain filling will be conducted to understand the impact on these families of molecules.

Tocopherols

The growing location has a significative impact on the total tocopherol content, but not on the individual tocopherols neither γ or δ-tocopherol (Table 2). Genotypes cultivated in the Lavaur and Airaines showed a significant lower content of total tocopherols (399.2mg/Kg of oil) than in the Poix-de-Picardie location (412.7mg/Kg of oil), in 2014.

Table 2. Sum of squares of combined analysis of variance for γ-tocopherol, δ-tocopherol and total tocopherol content in linseed for 10 varieties cultivated one year (2014) in 3 places: Airaines, poix-de-Picardie, and Lavaur. *** significant at p=0,001

	n	γ-tocopherol	δ-tocopherol	Total Tocopherol
Genotype (G)	10	848	3141280	**60705*** **
Location (L)	3	265	732764	**3627*** **
G x L	30	1573	5266731	**54481*** **

Year 2014 has a significative higher content of γ-tocopherol (p< 0.01) and tocopherol compared to 2013 and 2015 “(p<0,001) (Table 4).

There were also significative differences between genotypes in the same place for 2014 going from 331.7 to 473.7 mg/kg^{-1} of oil for the total tocopherol content and for γ-tocopherol from 329.6 to 466.5 mg/kg^{-1} of oil.

Phytosterols

Phytosterols content has showed a significative variation within sites and genotypes (Table 3). In The South there is a significative reduction of each individual phystosterol as well as the total. The Poix-de-Picardie location always presented the highest contents. Sterols are considered as plant hormones which play an important role in the protection of the plant against stress as well as in regulation of plant growth[2].

Table 3. Sum of squares of combined analysis of variance for individual phytosterols and total phytosterol content in linseed for 10 varieties cultivated one year (2014) in 3 places: Airaines, Poix-de-Picardie, and Lavaur. *** significant at p=0,001

	Campesterol	Stigmasterol	β-sitosterol	Avenasterol	Cycloartenol	Δ7-Stigmasterol	Total phytosterol
G	**13075*** **	**4818*** **	**53847*** **	**2830*** **	**11364*** **	1179	**206115*** **
L	**10824*** **	**741*** **	**55683*** **	**4845*** **	**4528*** **	**3469*** **	**312359*** **
G x L	**13086*** **	**3688*** **	**33872*** **	**3040*** **	**12770*** **	2170	**154026*** **

Year 2014 has a significative higher content of total phytosteols(p< 0.01) and β-sitosterol compared to 2013 and 2015 (p<0,001) (Table 4) but also for all the others phytosterols except Δ7-Stigmasterol.

There were also significative differences between genotypes in the same place for 2014 going from 192.3 to 519.6 mg/100g^{-1} of oil for the total phytosterol content, for β-sitosterol from 67.0 to 95.7 mg/kg^{-1} of oil and for cycloartenol from 36,9 to 154.4 mg/kg^{-1} of oil.

Table 4. Mean values for all the cultivars in all locations per year

Cultivars	Oil content (g/g DM)	Tocopherols (mg·Kg^{-1} oil)		Phytosterols (mg·100 g^{-1} oil)	
		Gamma	Total	β-sitosterol	Total
2013	0.48	391.2	397.6	127.0	347.4
2014	0.49	409.8	425.2	128.3	384.9
2015	0.46	362.6	364.6	109.5	307.0

CONCLUSION

This study shows that tocopherol and phytosterol contents in linseed oil depend on genetic and environmental factors. High temperatures reduce the content of tocopherols and phytosterols. This could be due to a protective role of tocopherols and phytosterols as antioxydants against hot stress, leading to a decrease of their content in the plant. The variability encountered within varieties allows the possibility to increase the content of these two families of components by breeding, finding varieties with a good nutritional or cosmetic profile that could preserve oil quality for a longer period.

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