XV INTERNATIONAL LUPIN CONFERENCE 2019

ESTIMATES OF TOTAL PHENOLICS CONTENT IN PERUVIAN ECOTYPES OF LUPIN (*Lupinus mutabilis*) (BITTER AND DEBITTERED)

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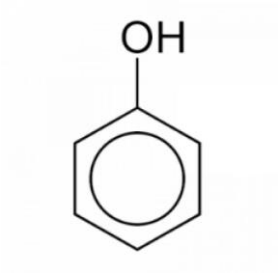
Objective

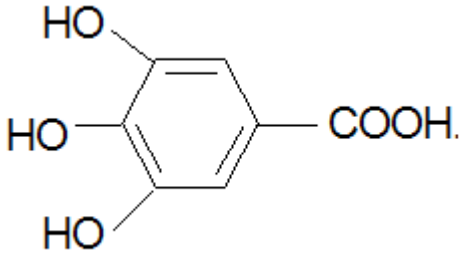
- Characterize the total phenolic content in 52 different Ecotypes of Peruvian Lupinus mutabilis.
- Evaluate the effect of water washing processing (for alkaloid removal) in the phenolics content that remains in ecotypes.
- Evaluate alternative methodologies in the phenolics content determinations, that could help in the characterization of thousand of ecotypes for Peruvian biodiversity. For example methods based in spectroscopy.

Benefit of Phenolic Compounds.

Beneficial effects of phenolic compounds:

- Antioxidant activity
- Estrogen antagonism
- Antiangiogenic effect.
- Promotion of apoptosis
- Inhibition of Cyt. P450, protein kinases and telomerases.





Phenolic Compounds in plants

- They are powerful inhibitors of HNO_2 (nitrous acid) which decomposes nitrosamines and causes deamination of DNA bases, especially guanine.
- Phenolic compounds protect the gastrointestinal system in situations of excessive production of reactive nitrogenous species

Fuente: Cadenas y Packer, 2002. Handbook of Antioxidants. Second Edition. CRC Taylor y Francis.

Materials and Methods

For this research, 52 ecotypes of Lupinus mutabilis (called “Chocho” or “Tarwi” in Peru) were obtained as seeds.

TD1		Altagracia
TD2		Compuesto blanco semi precoz
TD3		Cholo fuerte
TD4		Andenes INIA
TD5		Huánuco 2
TD6		Patón grande
TD7		Lircay – Huancavelica
TD8		Huancavelica (Sihuas)
TD9		Puno 2
TD10		Puno 1
TD11		Cajamarca
TD12		Moteado beige
TD13		Cheje Copani blanco

TD14		H6 INIA
TD15		Yanamuclo 008-2
TD16		Yunguyo
TD17		Yanamuclo PLGO
TD18		Huánuco 1
TD19		Andenes – 80
TD20		Pallasca
TD21		Churibamba Huánuco 4384
TD22		Comercial (mezcla)
TD23		Chacas
TD24		H6 INIA plomo
TD25		CD Junín 1

TD26		CD Junín 2
TD27		CD Junín 3
TD28		CD Junín 4
TD29		CD Junín 5
TD30		CD Junín 6
TD31		CD Junín 9

TD31		CD Junín 9
TD32		SGC 22
TD33		CD Junín 10 -1
TD34		Yunguyo beige
TD35		Andenes UNALM
TD36		Yunguyo negro

TD37		Cheje Copani negro
TD38		Tauribamba (Sihuas) -1
TD39		CD Junín 7- 1
TD40		CD Junín 7 -2
TD41		Abancay
TD42		Vicos

TD43		Tauribamba blanco
TD44		CD Junín 10-2
TD45		Huallanca blanco
TD46		H6 INIA blanco
TD47		Yana tarwi
TD48		Yanamuclo 008-3

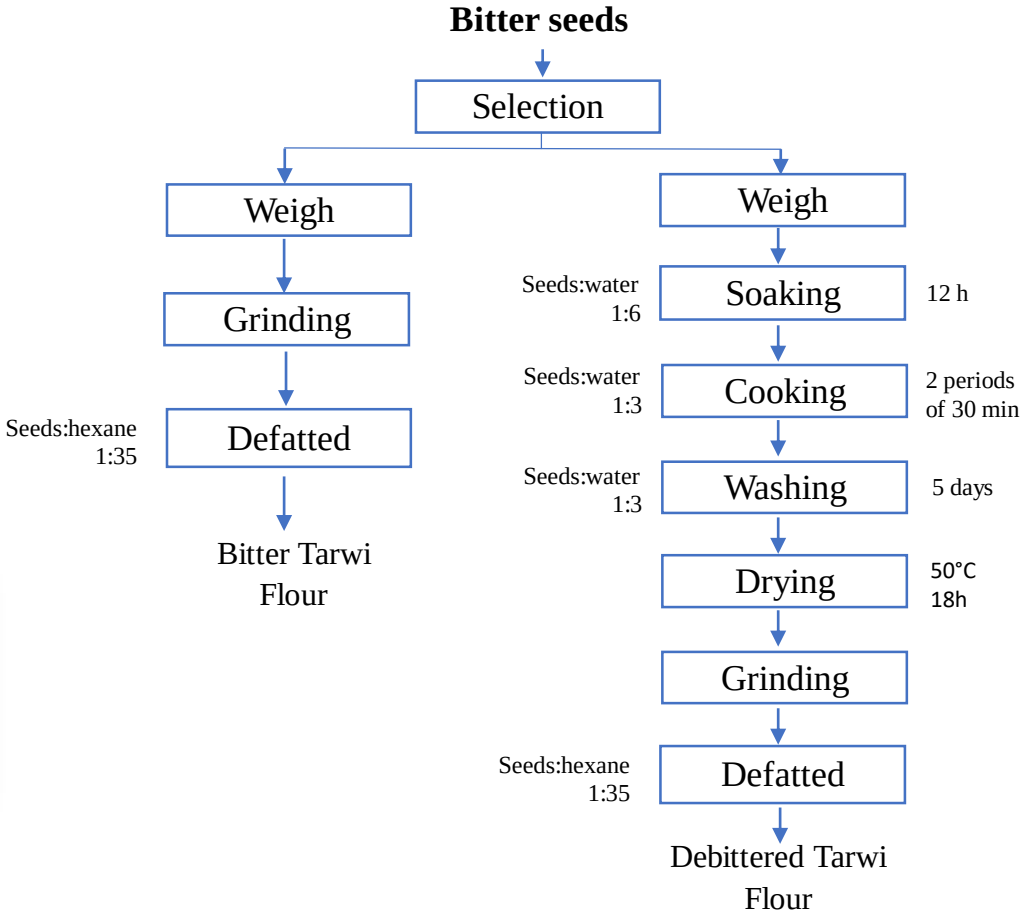
TD49		Tauribamba (Sihuas)-2
TD50		Tauribamba (Sihuas)-3
51		PLGO 3
52		Huánuco 1 B/N

52 Ecotypes studied

Provided by: Legume Program at UNALM Peru

Fuente: Fotos tesis Ingeniero UNALM 2019 Autor Zuly Sabelino-Francia

These seeds were stored in polietilene bags under refrigeration at 5 °C until analysis.

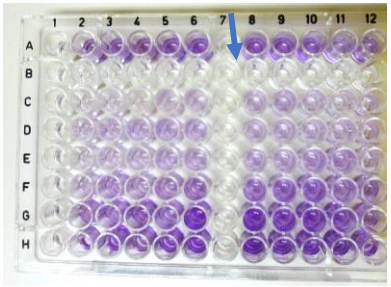


Total Phenolics colorimetric determination

Phenolics extraction in 50% ethanol solution as a solvent

Defatted tarwi flour (g): solvent (ml) 1:20. Sonicated twice in an ultrasound bath, period = 30 min.

samples were centrifuged at 3000 RPM for 15 minutes at 5 ° C.



The quantification of the phenolic content was performed by the Folin Ciocalteau

Microplate format: readings at 726 nm

Quiroz-Reyes, C., M. Aguilar-Méndez, et al. (2013). "Comparative study of ultrasound and maceration techniques for the extraction of polyphenols from cocoa beans (Theobroma cacao L.)." Revista mexicana de ingeniería química **12**(1).

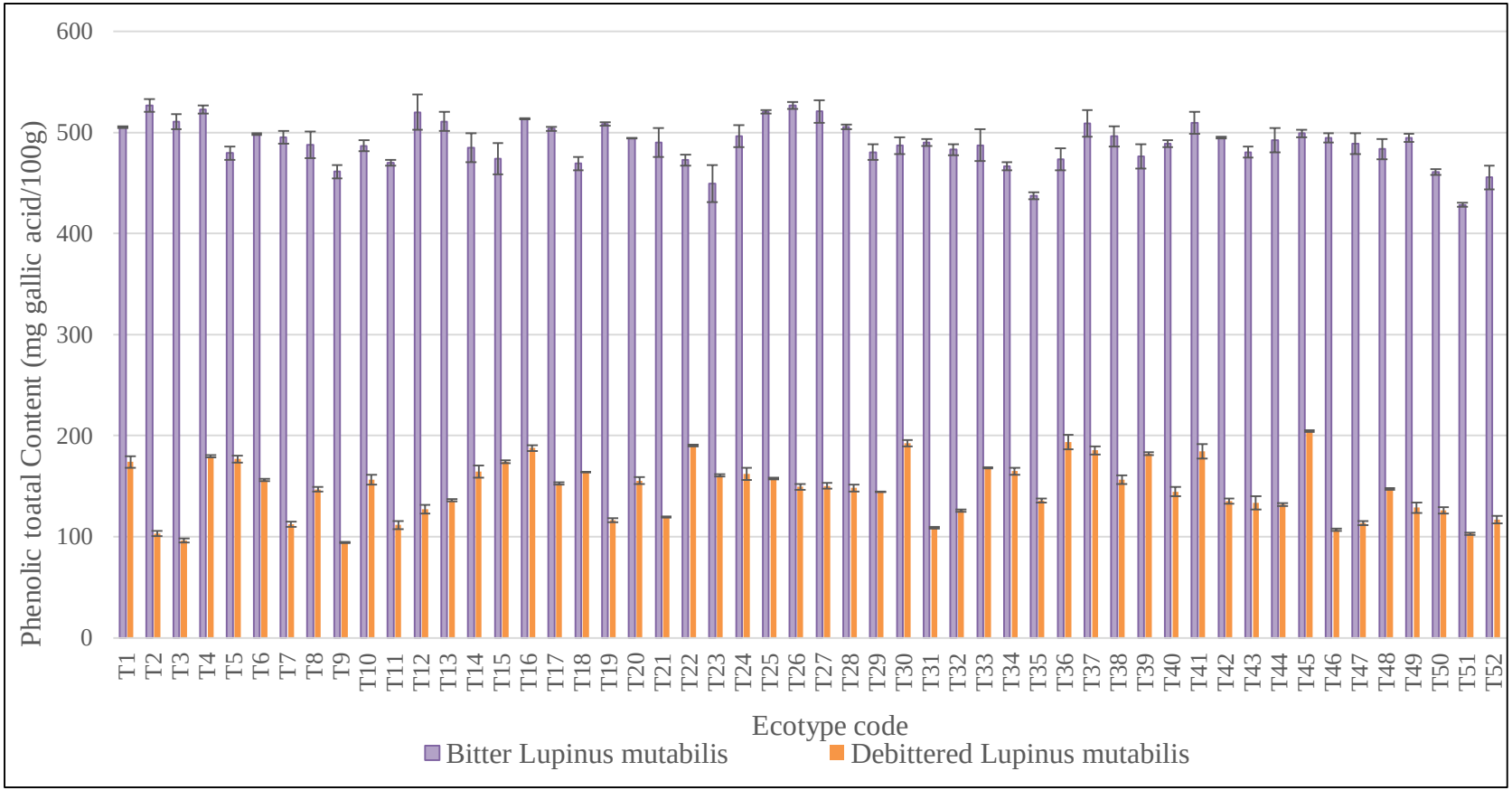


Figure 1. Phenolic Total Content in bitter and debittered *Lupinus mutabilis*

Fuente: tesis Ingeniero UNALM 2019 Autor Zuly Sabelino-Francia

Table 1. Average phenolic total content in genotypes of *Lupinus mutabilis*

	Bitter*	Debittered*
Mean	489.71	147.31
Minimum	428.49	94.45
Maximum	526.80	204.67
Standard deviation	21.66	28.61

70% reducción (Effect of water washing debittering)

35.8%

61.2%

* mg gallic acid / 100g sample

For low Phenolics content genotypes, values of reductions simlar to earlier reports of Jimenez-Martinez et al 2007

	Ecotype	Origin
Min	PLGO 3	Junín
Max	Compuesto blanco semi precoz	Junín

Fuente: tesis Ingeniero UNALM 2019 Autor Zuly Sabelino-Francia

Construction of MIR spectrum

For methanolic extracts and flours.

Other possibility for phenolics determinations….and other determinations

Use of spectroscopy en the stimation of the composition of Tarwi (Including phenolics)

- Construction of IR spectrum in the MIR Region.
- Use of FTIR spectromenter Portable. Thermo

Atenuatted Total Reflectance- Fourier Transform Infrared (ATR- FTIR) spectrometer (Tru Defender TM- Thermo Fisher).

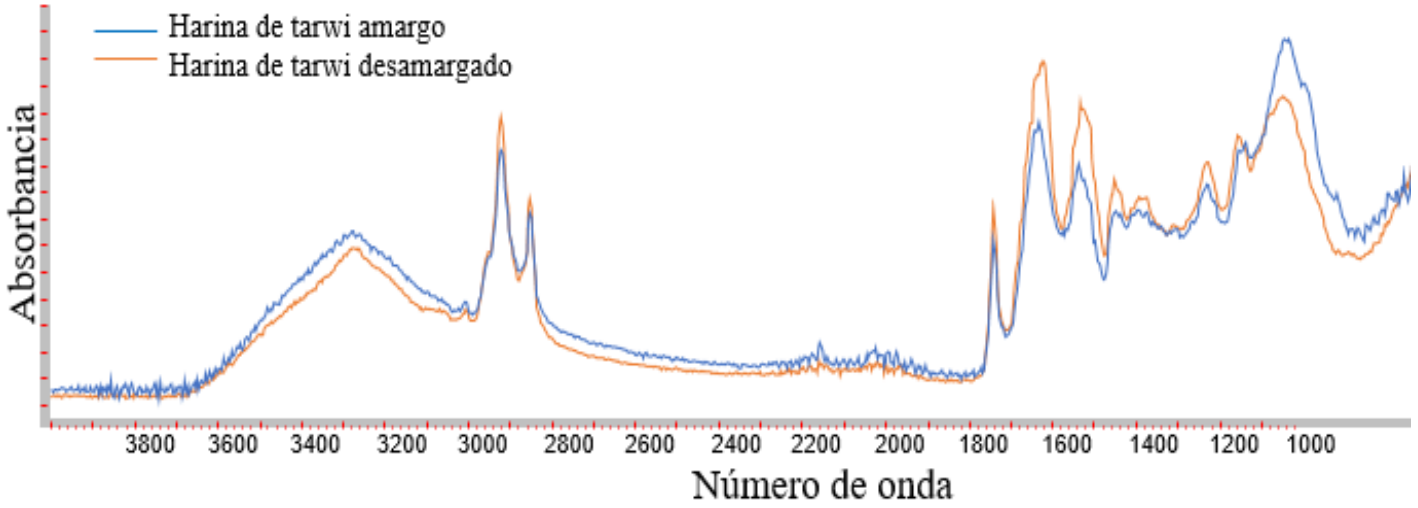
Courtesy Dr Luis Rodriguez-Saona.

Read the spectrum of Lupin Flour…

Whole Lupin Flour

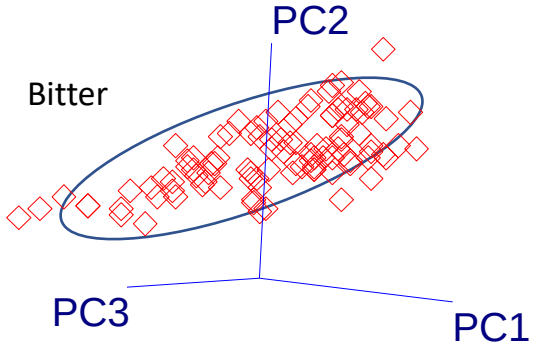
Spectrometer Tru Defender TM- Thermo Fisher

MIR Spectrum characteristic of *L. mutabilis* flour

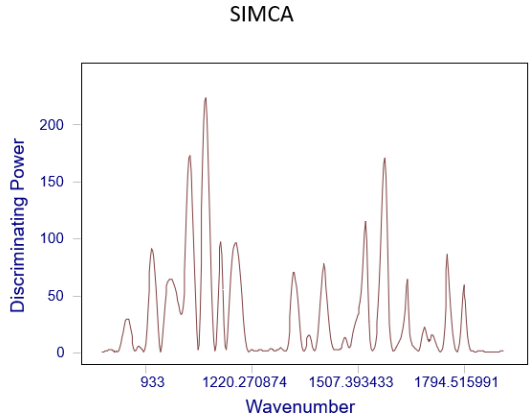


Fuente: tesis Ingeniero UNALM 2019 Autor Zuly Sabelino-Francia

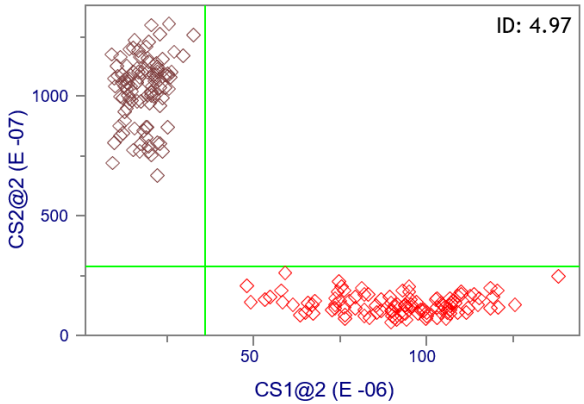
Fast classification between bitter and debittered tarwi flour.



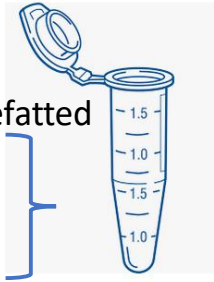
Discriminating Power



SIMCA Clasification
Soft Independence modeling of class analogy



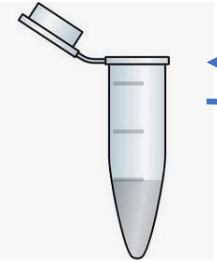
Fast method phenolics using FT IR spectrum



0.1 g of defatted flour

0.5 ml of methanol

Ultrasound



After centrifugation…

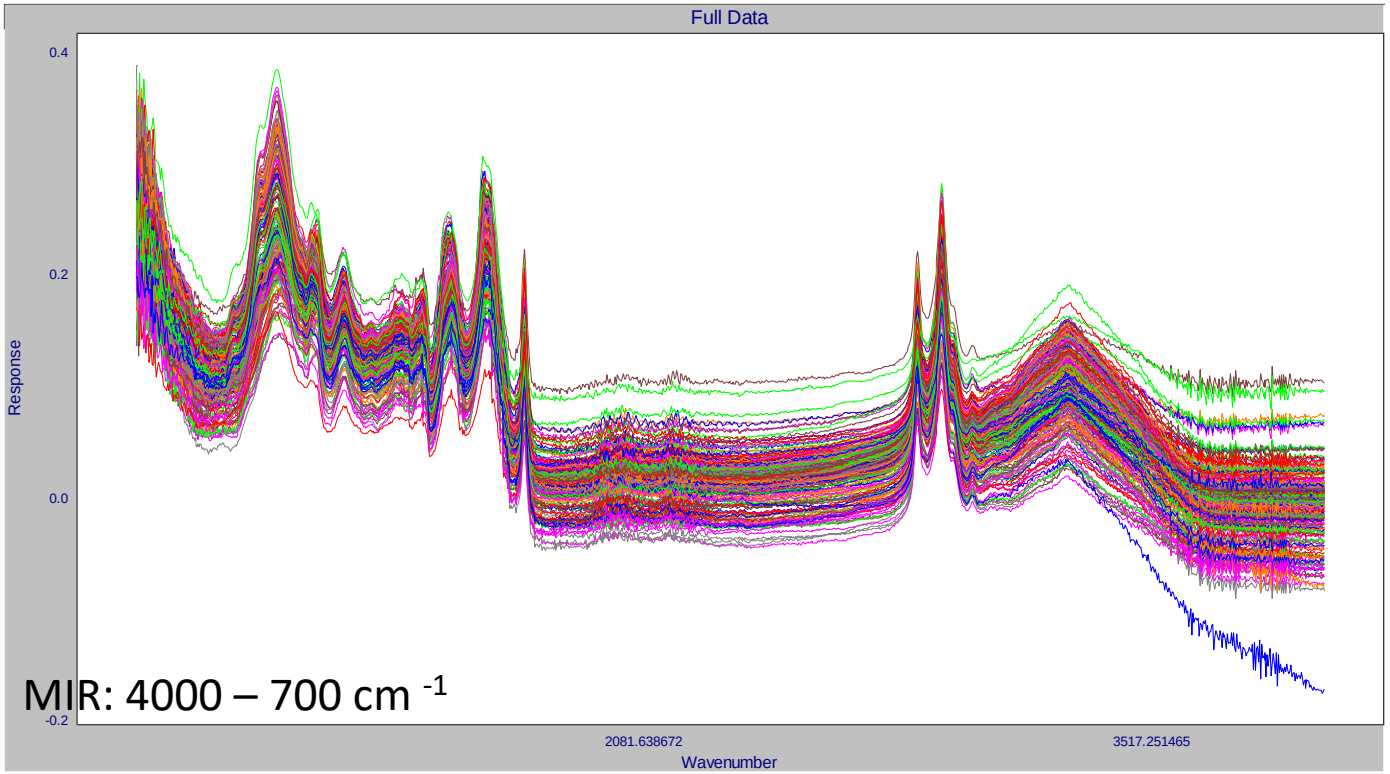
Let evaporate 20 ul extract over ATR sensor

Collect FT IR Spectrum

Chemiometrics software: multivariate statistical análisis. We used Pirouette (v 4.0, Infometrix, USA)

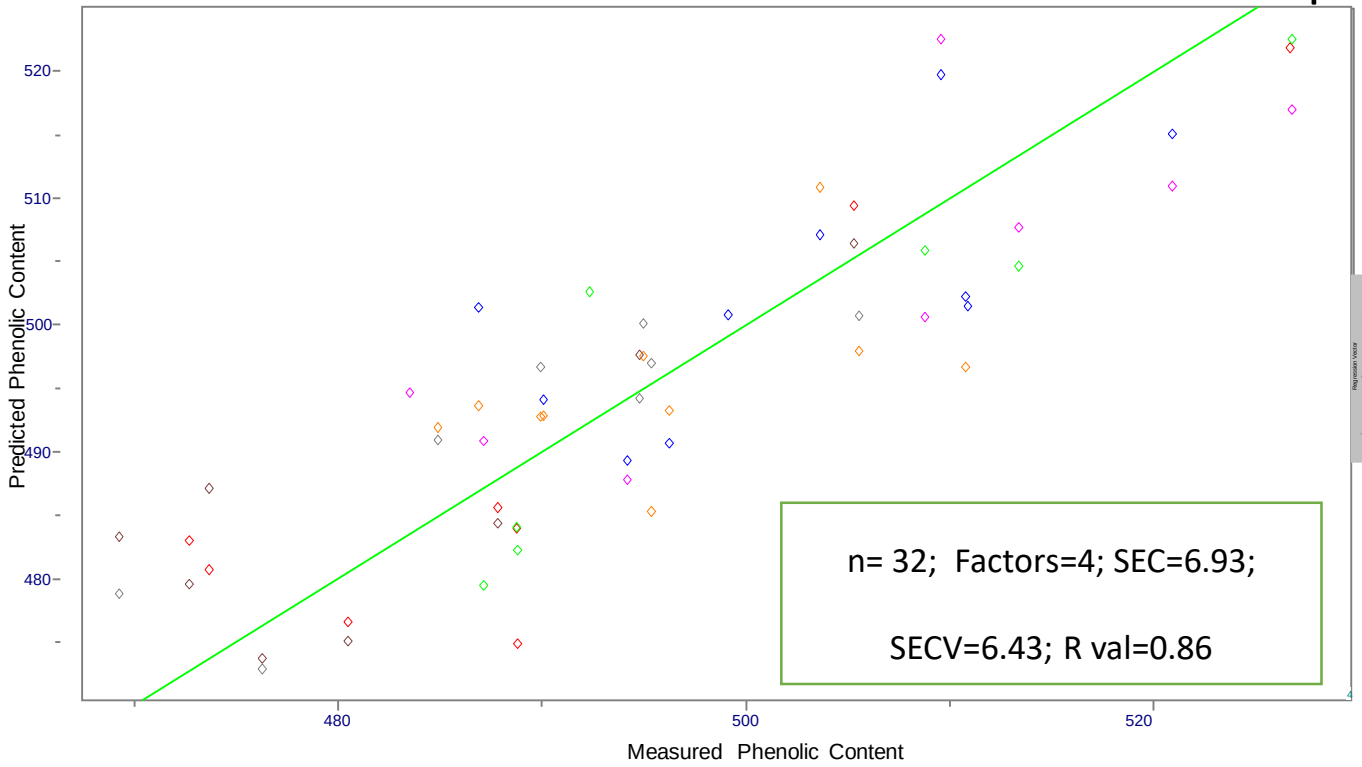
FT- IR spectrum for 52 genotypes (methanolic extracts)

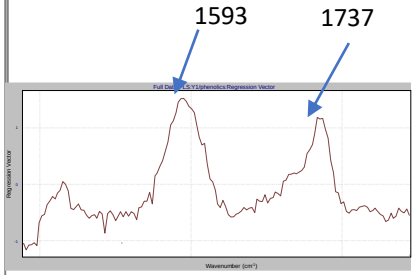
Ongoing work for the fast determinations of phenolics based on FT IR spectrum



Fuente: tesis Ingeniero UNALM 2019 Autor Zuly Sabelino-Francia

Preliminary results for calibration phenolics extract with FT-IR MIR……..needs improvement





Phenolics regression vector

Partial Least Square regression (PLSR) plot for Total Phenolic content using MIR region (1837- 1421 cm^{-1})

Conclusions

- The 52 bitter genotypes explored showed a variability in content of phenolics of 21.6%. Values ranged between 428 and 526 mg Ac galico/100 g.
- Phenolic content decreased for all ecotypes analyzed after debittering. The type of phenolic that remains after debittering needs to be analyzed.
- The FT-IR spectroscopy to be improved would allow the characterization of high numbers of material in germoplasm Banks.
- FT-IR spectroscopy confirms important changes in composition between bitter and unbitter siamples that allows a rapid clasification of lupin flours.

Aknowledgements

- We acknowledge Dr. Luis Rodriguez-Saona for kindly provide the portable FT-IR (ATR-MIR Thermo) and to the PNIA project for Tarwi directed by Felix Camarena Mayta for funding.