

THE CASCADE BIOREFINERY APPROACH FOR THE VALORIZATION OF THE SPENT COFFEE GROUNDS

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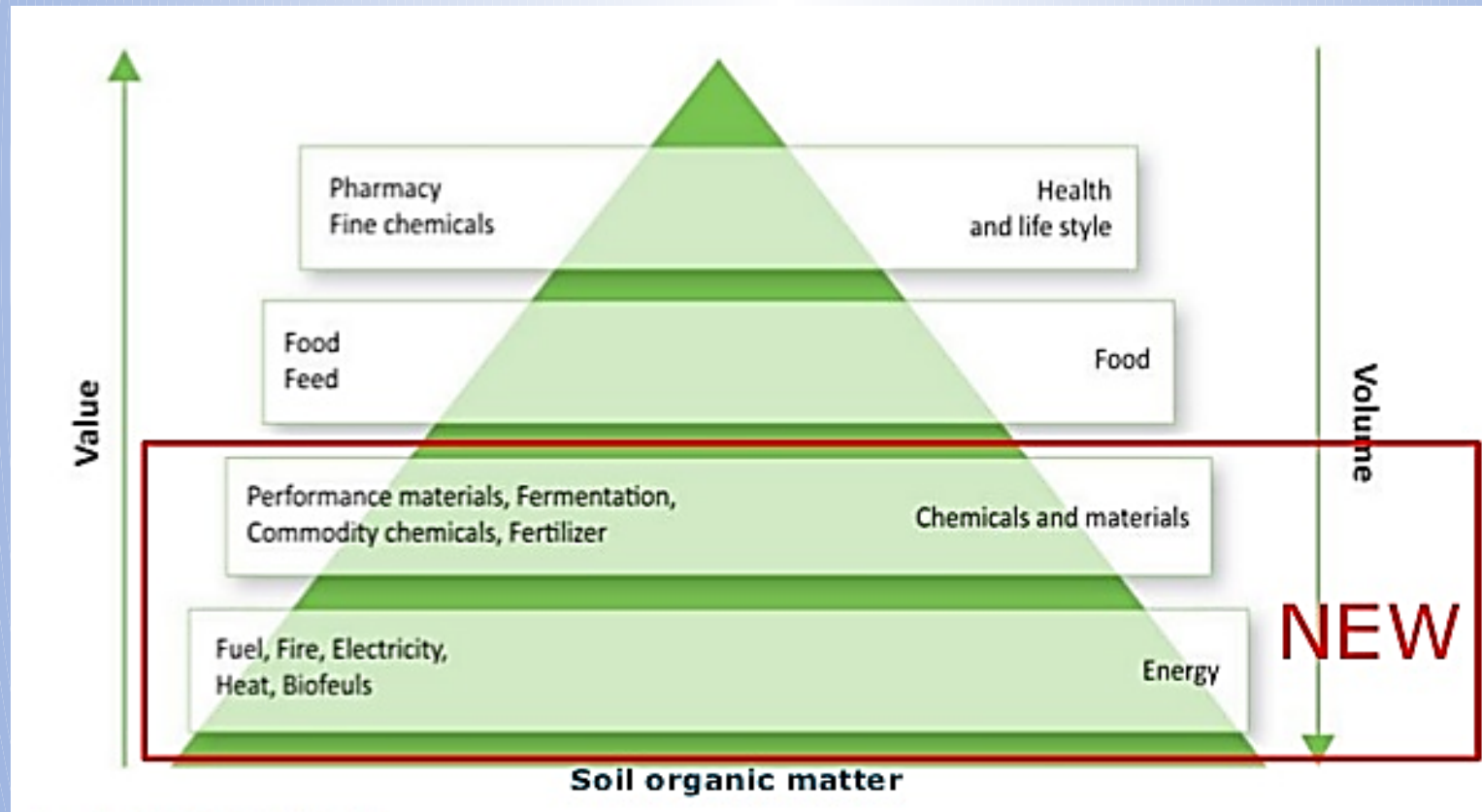
Coffee is one of the most appreciated beverage around the world with a global annual consumption of 9.3 tons.

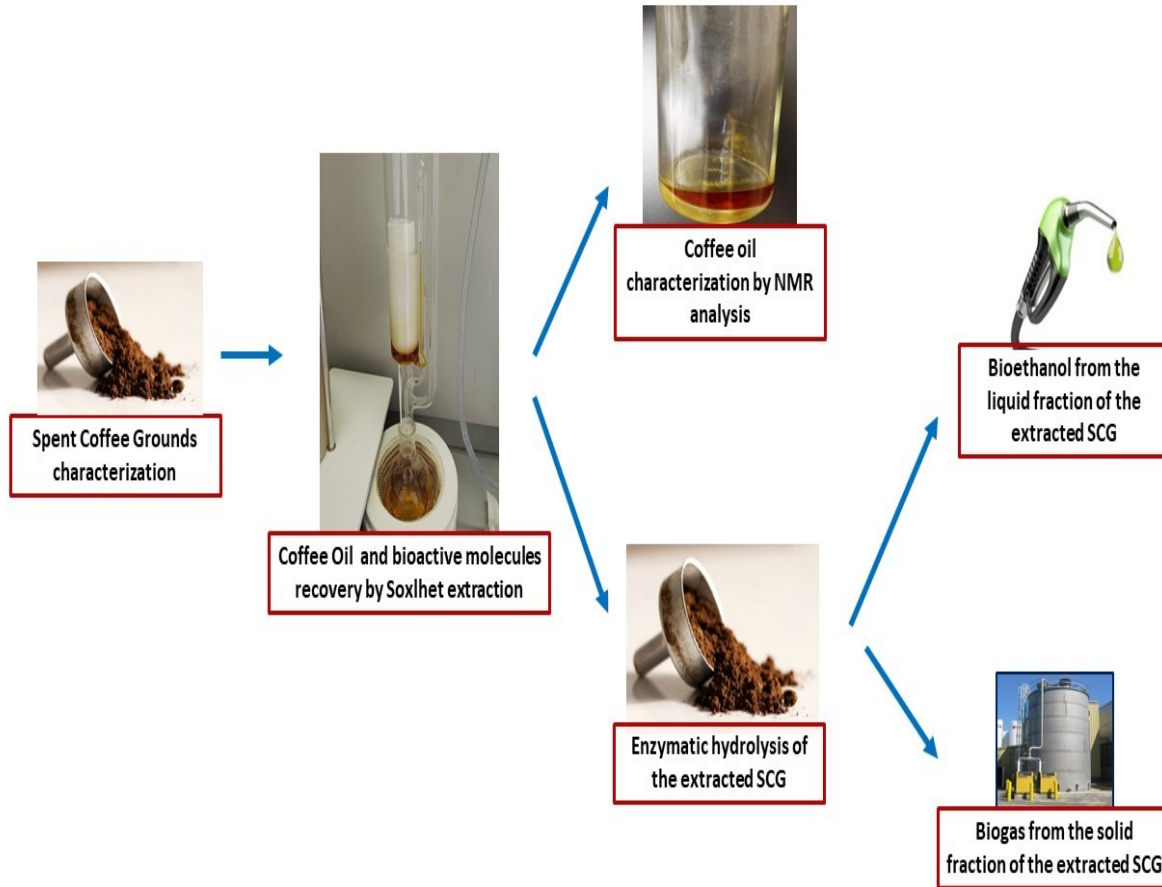
Spent Coffee Grounds (SCG), annual amount of 6 tons generated at international level.

Currently SCG are mainly incinerated or simply collected with the Organic Fraction of the Municipal Solid Wastes (OFMSW) and disposed in landfill.



The new EU Waste Framework Directive promotes the
“cascade biorefinery approach” for agrofood wastes





Based on this configuration, SCG will be used for:

- the extraction of **high economic value molecules** (tocopherols, linoleic acid, chlorogenic acid, Cafestol and Kahweol);
- **bioethanol production** by *Saccharomyces Cerevisiae* fermentation with a preliminary stage of combined acid-enzymatic hydrolysis;
- **biogas production** by AD.

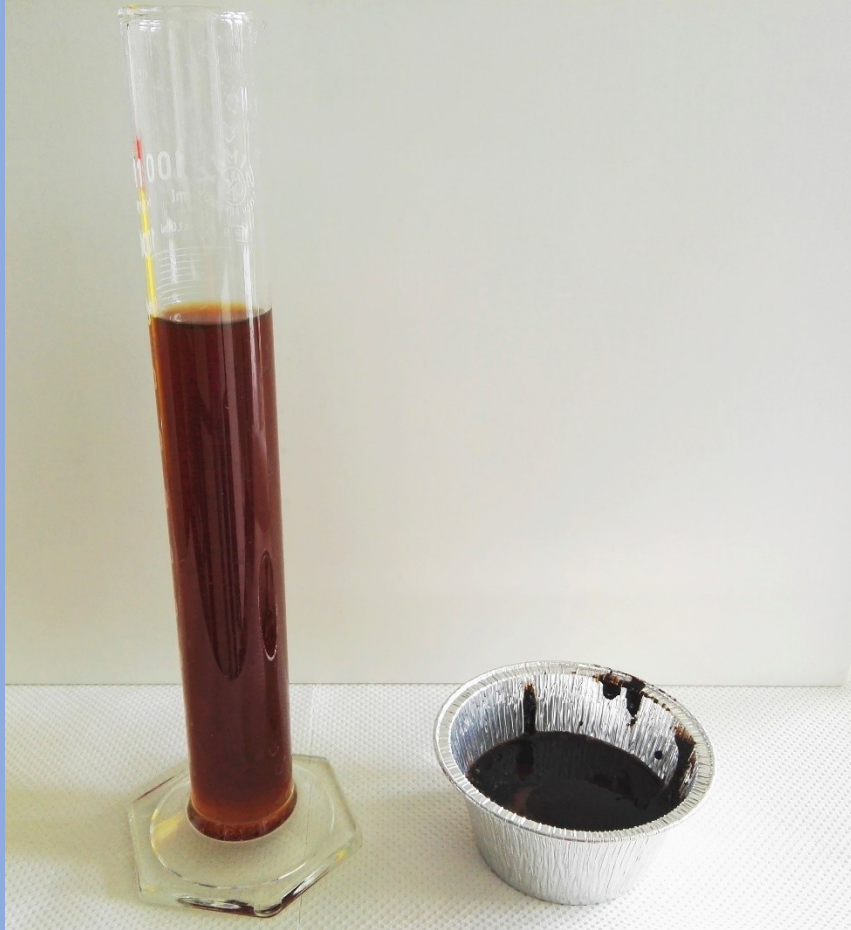
Coffee oil extraction

10 g of dried was located in a Soxhlet extractor.
300 mL of polar and no-polar solvents (acetone, ethanol, iso-propanol and n-hexane and a 50:50 (v/v) mixture of the ethanol and iso-propanol, were tested).

All the solvents were tested at 85°C, value higher than boiling temperature of the different solvents.



$$COE (\% w/w) = \frac{M_{oil}}{M_{SCG}} \times 100$$



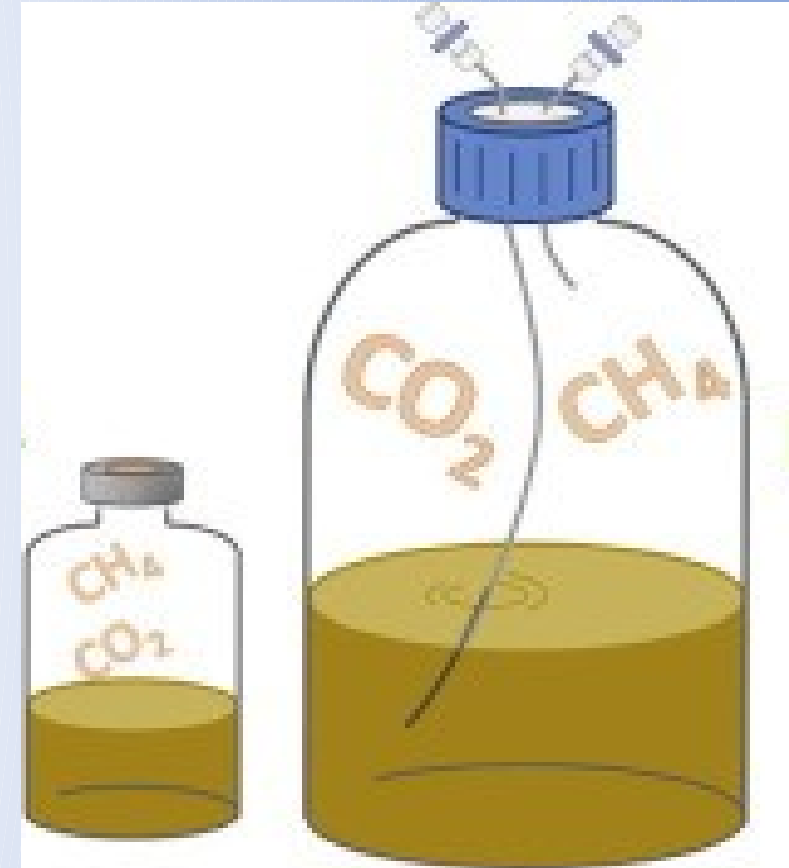
Enzymatic hydrolysis and bioethanol production

The extracted SCG were performed through an acid pretreatment by 1% H₂SO₄ addition. After 1 hour, the pH was adjusted at 5.5 for the enzymatic hydrolysis adding a 2% w/w of Cellic CTec-2 (Novozymes) cellulase. The enzymatic hydrolysis was performed at 55°C and had a duration of 48 h.

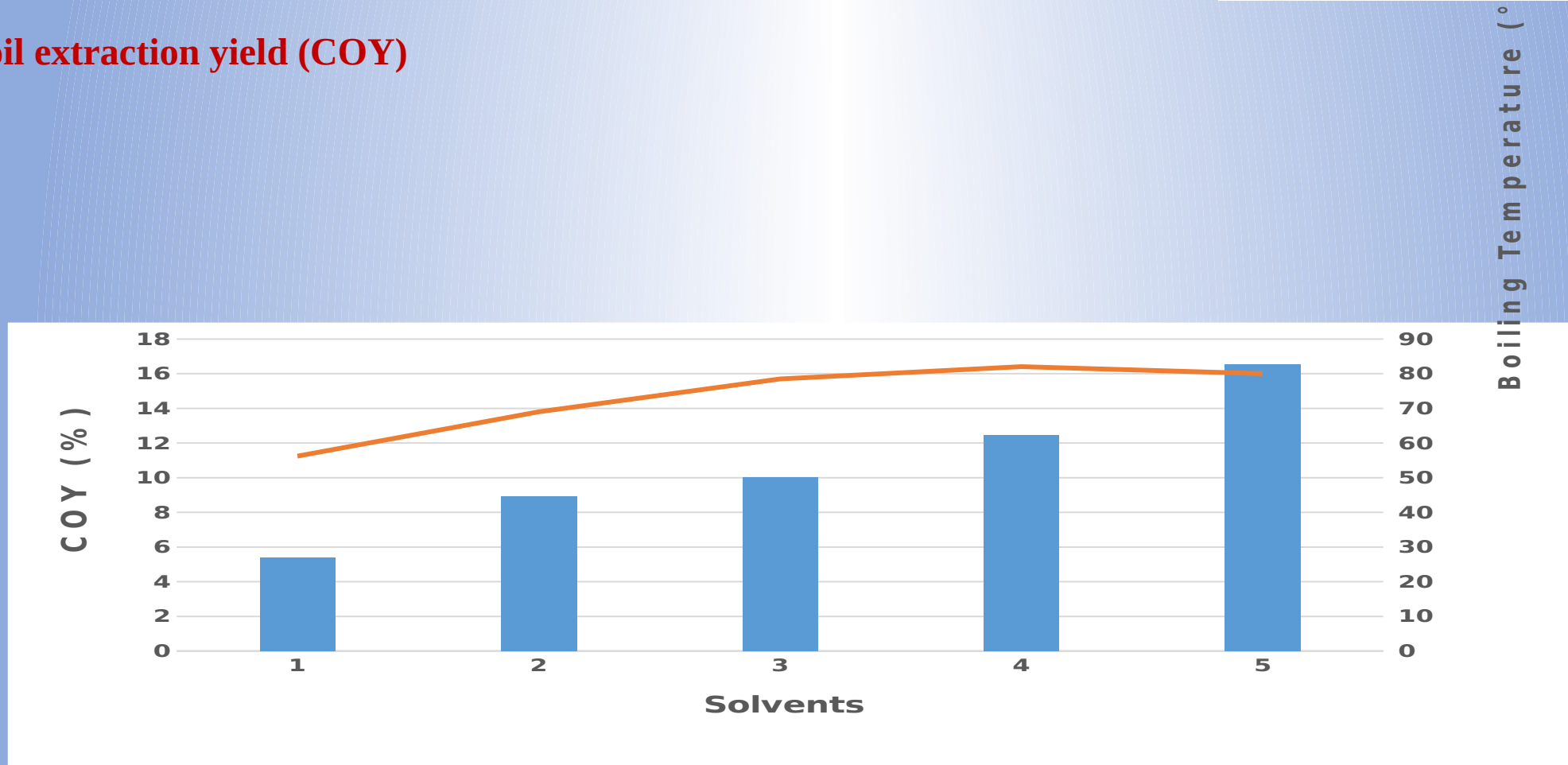
At the end of the enzymatic hydrolysis the SCG were centrifuged at 5,000 rpm for 20 minutes. The liquid fraction, rich in soluble sugars, has been fermented by *Saccharomyces Cerevisiae* anaerobic batch fermentation at 37°C.

Biogas production from the solid fraction

Solid fraction (SF) from acid-enzymatic hydrolysis was performed for the biogas production by AD in mesophilic conditions.



Coffee oil extraction yield (COY)

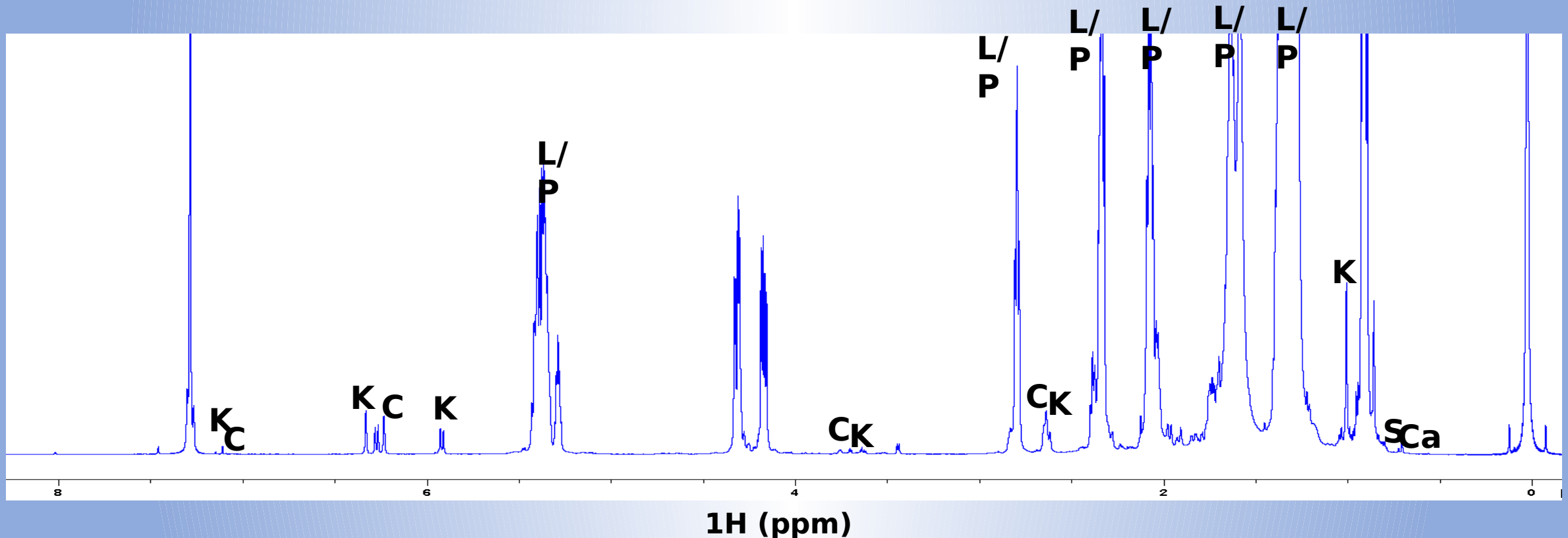


n-hexane is characterized by high value, $K_{ow} = 4.5 \times 10^4$, revealing its hydrophobic nature.

Instead, iso-propanol ($K_{ow} = 0.64$) and ethanol ($K_{ow} = 0.54$) have partition coefficients close to one, which means that these solvents, even if are more hydrophilic, are also able to extract hydrophobic components during Soxhlet extraction

Coffee oil compounds

NMR spectroscopy was used to determine the main compounds present in coffee oil. **Linoleic acid, Cafestol and Kawheol**, appear as esters of fatty acids, and sterols such as Stigmatasterol, and Campesterol in **hexane extraction**.

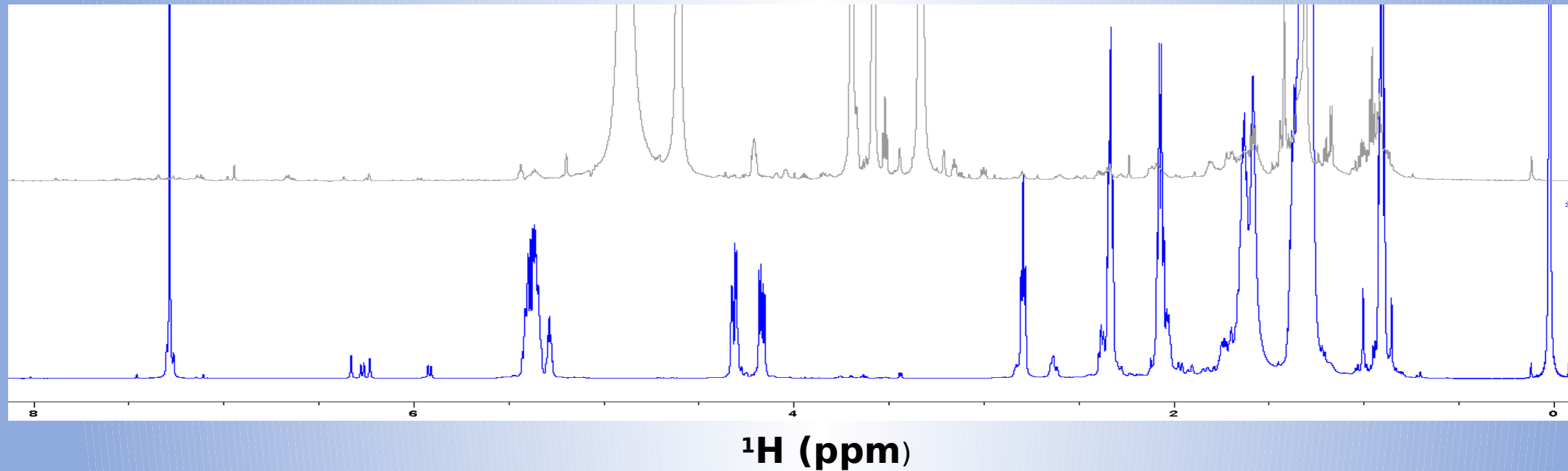


¹H NMR spectra of SCG extract by Soxhlet extraction

The following signals are marked: kahweol (K), cafestol (C), linoleic acid (L), Sitosterol and Stigmatasterol (S), Campesterol (Ca)

Coffee oil compounds

^1H NMR spectra of coffee oil extracted by **hexane (blue)** or by **ethanol/isopropanol mixture (50:50 v/v) (grey)**.

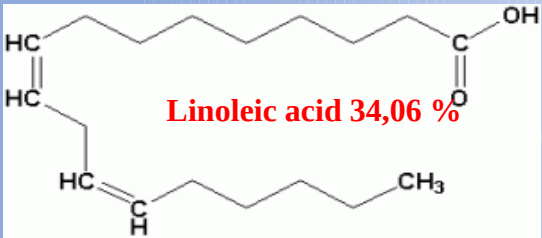
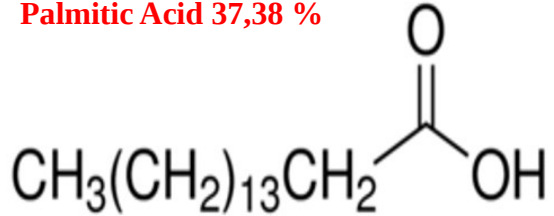


Both extracts contain Cafestol, Kahweol and linoleic acid. However, only small amounts of linoleic acid are extracted with the polar solvents mixture (lower signals in the range 0-3 ppm), while the Cafestol and Kahweol signals had higher intensities (3-4 ppm). The use of ethanol/isopropanol mixture to the process allows the process to remain green

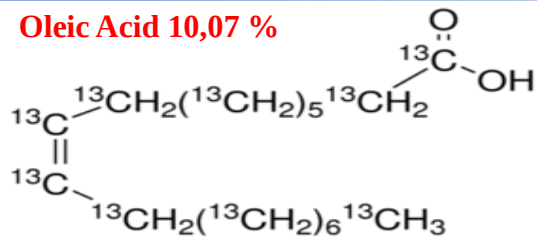
Coffee oil compounds

Fatty Acids

Palmitic Acid 37,38 %



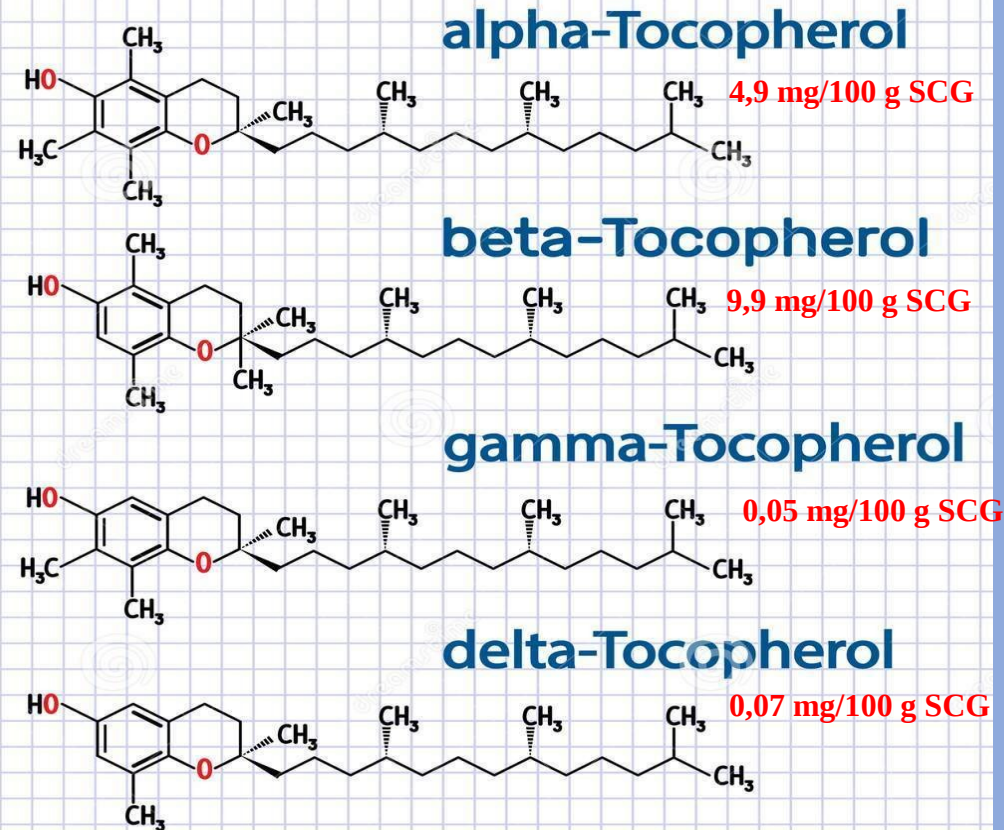
Oleic Acid 10,07 %



Tocopherols

About 15 mg/100 g SCG

Literature range: 6,5-30 mg/100 SCG



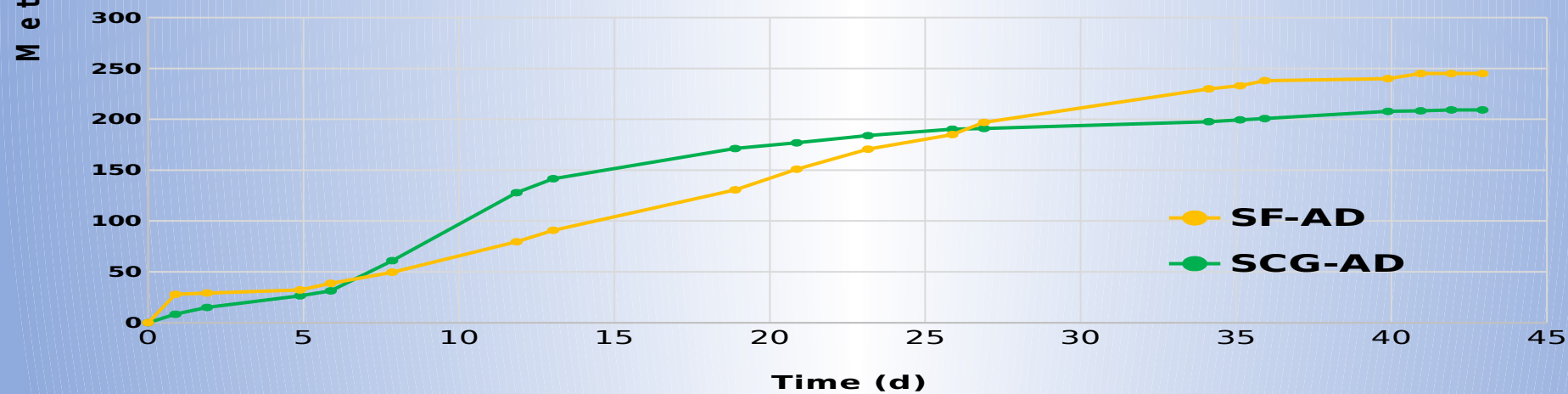
Enzymatic hydrolysis and Ethanol Production

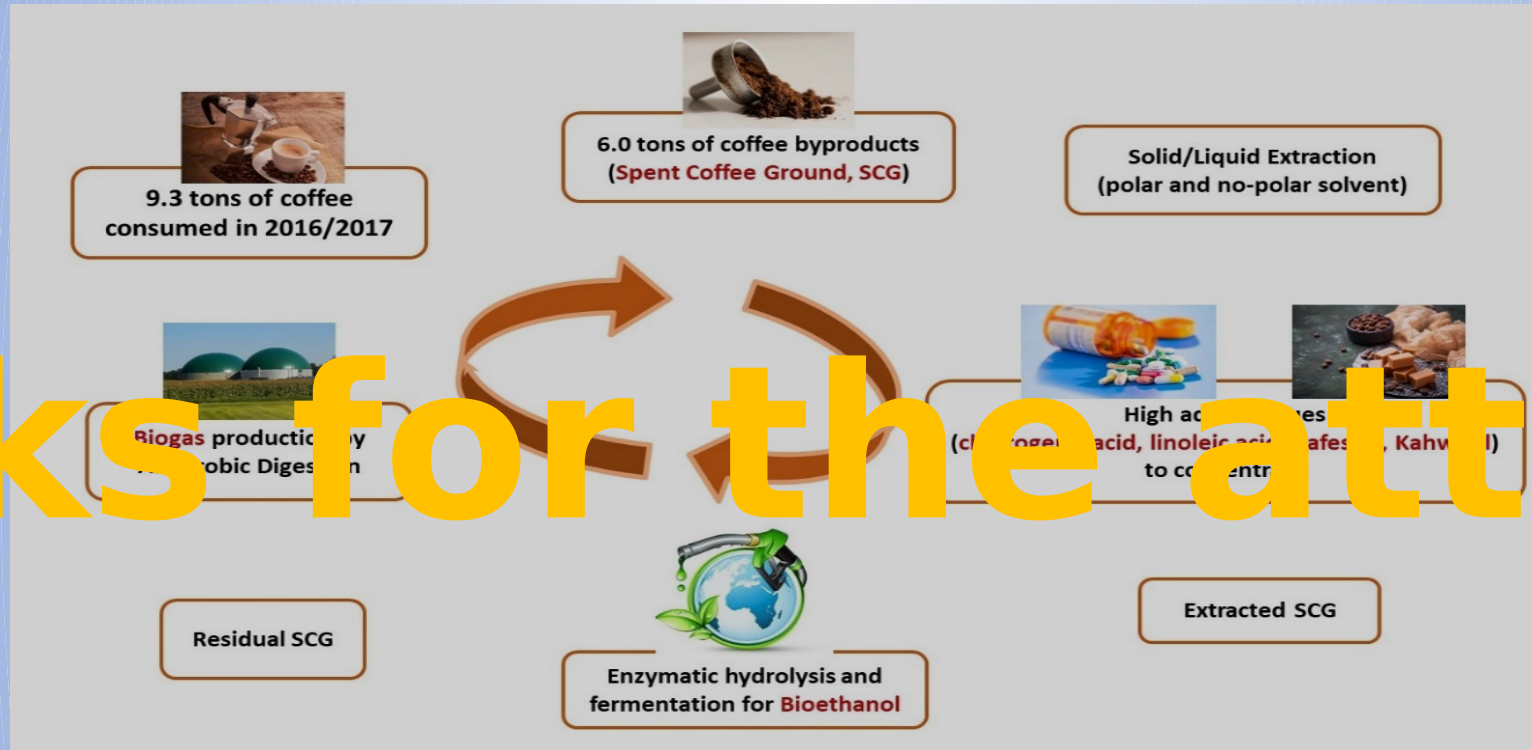
	Glucose (g/L)	Ethanol (g/L)
SCG EtOH tests	95.65 ± 12.60	46.66 ± 4.40
L-EtOH tests	103.29 ± 5.84	49.12 ± 7.48
SL-EtOH tests	100.27 ± 3.91	45.99 ± 8.28

These results allowed achieving **two important conclusions**. The first is that **bioethanol production was not influenced by coffee oil extraction**.

The second conclusion is that liquid phase after acid-enzymatic stage contained the soluble molecules, hexose sugars, exploitable by *S. Cerevisiae* for bioethanol production. Instead, solid phase brought to a slight reduction of bioethanol production (Table 2), probably as effect of a minimal glucose absorption of solid phase.

Biogas Production from solid phase



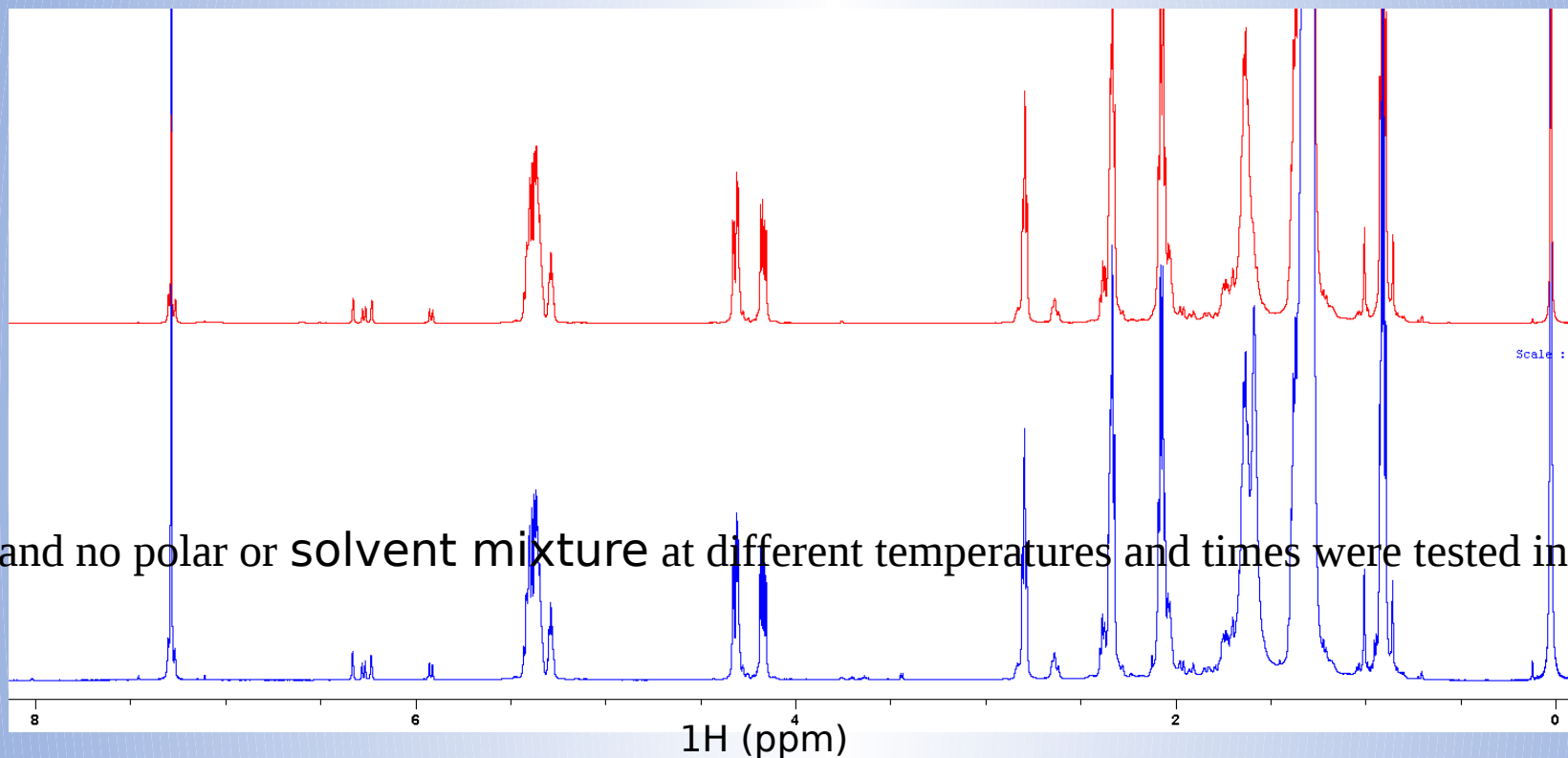


Thanks for the attention

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Different polar and no polar or solvent mixture at different temperatures and times were tested in a Soxhlet apparatus

^1H NMR spectra of extracted from SCG in hexane with (blue) or without (red) the Soxhlet apparatus

A similar recovery of valuable compounds was also found for extraction without or after one cycle through the Soxhlet extraction.

No presence of compounds was observed after more (quanti ne avevi fatti? E a quanti gradi ?)