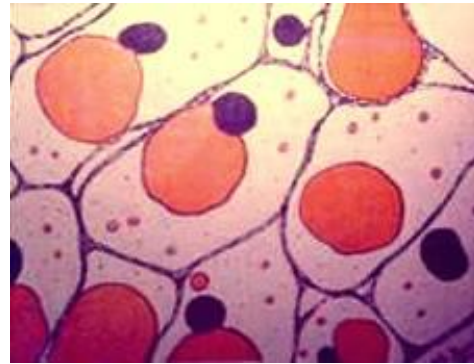
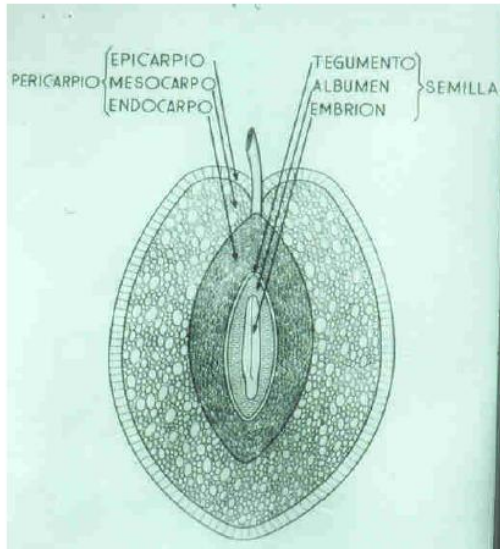




Use of Enzymes in Olive Oil production

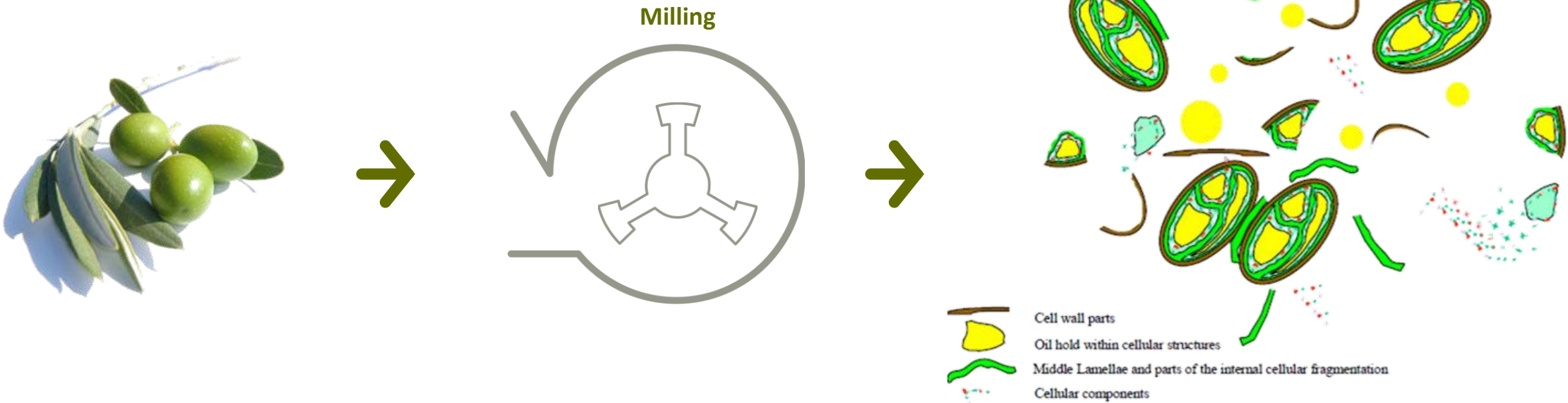
Rémi Lévêque
Dr. Gunter Maier
October 2017

Crushing – cell preparation



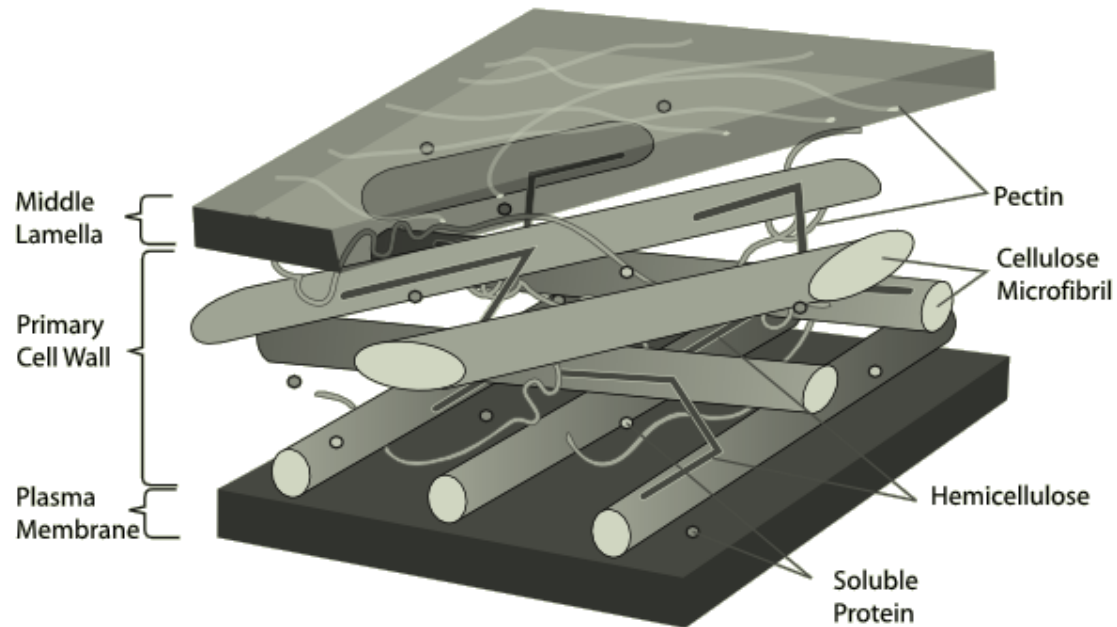
comments

Milling - Mechanical effect



Olives Cell-wall are break down in small particles at milling stage.
The Oil is hold within the cellular structure and further extracted by decanter

The chemistry behind Olive Oil processing with enzymes



1: HYDROLASES

Catalyse reactions between a substrate and water and bind water to certain molecules. In this way larger molecules are broken up into smaller units

e.g. Amylases, Cellulases, Lipases, PME and PG, Proteases, Pullulanases

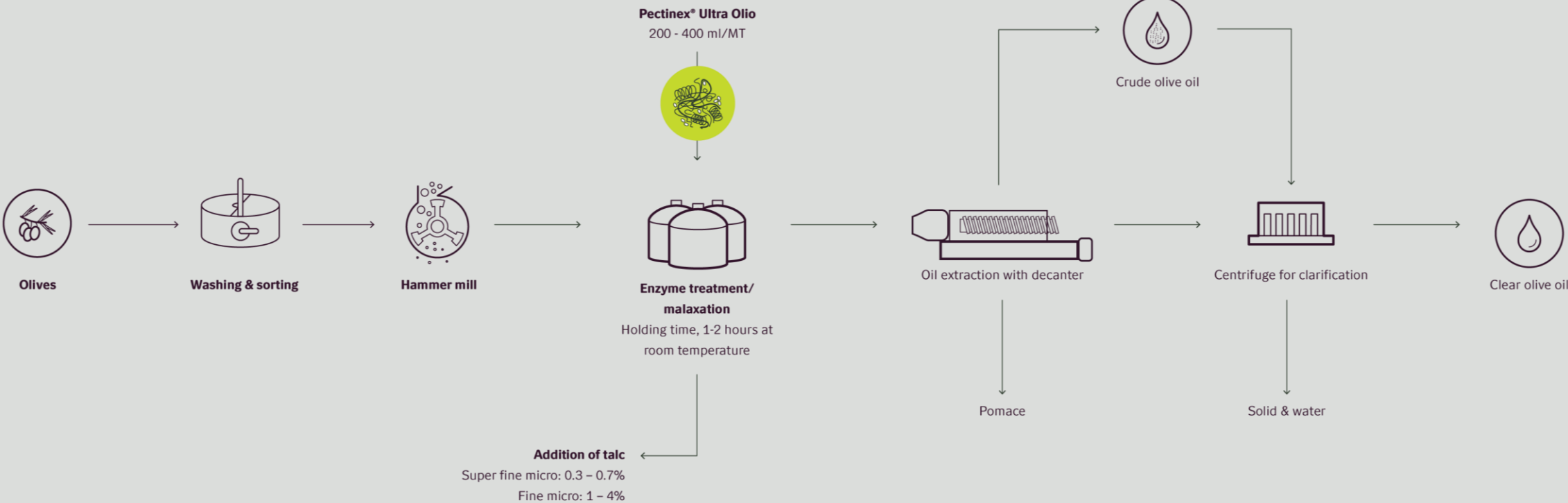
2: LYASES

Catalyse the addition of groups to double bonds or the formation of double bonds through the removal of groups. Thus bonds are cleaved using a different principle to hydrolysis

e.g. Pectin lyase, Pectat lyase, Alpha-actetolactate decarboxylase

Olive oil production

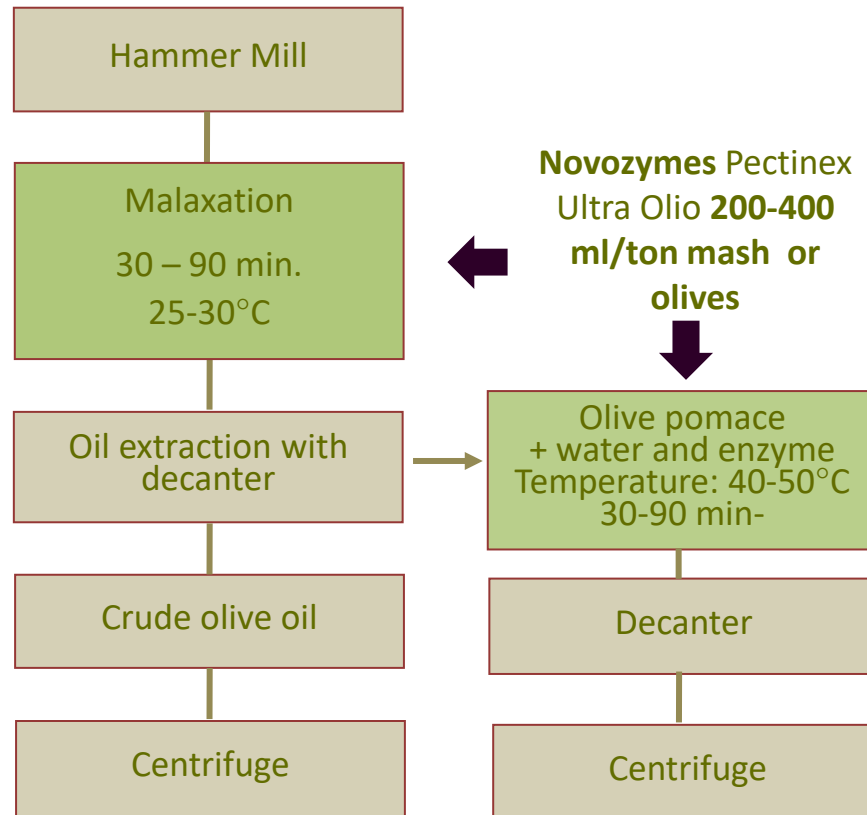
Clear and stable olive oil production with Novozymes Pectinex® Ultra Olio



Olive oil production

Clear and stable olive oil production with Novozymes Pectinex® Ultra Olio

Option:
Addition of talc,
Super fine micro:
0.3-0.7 %
Fine micro: 1 - 4 %



- In **discontinuous** lines, a diluted enzyme solution - 10% in cold, clean tap water - is added directly into the mill and divided into 3-4 portions.

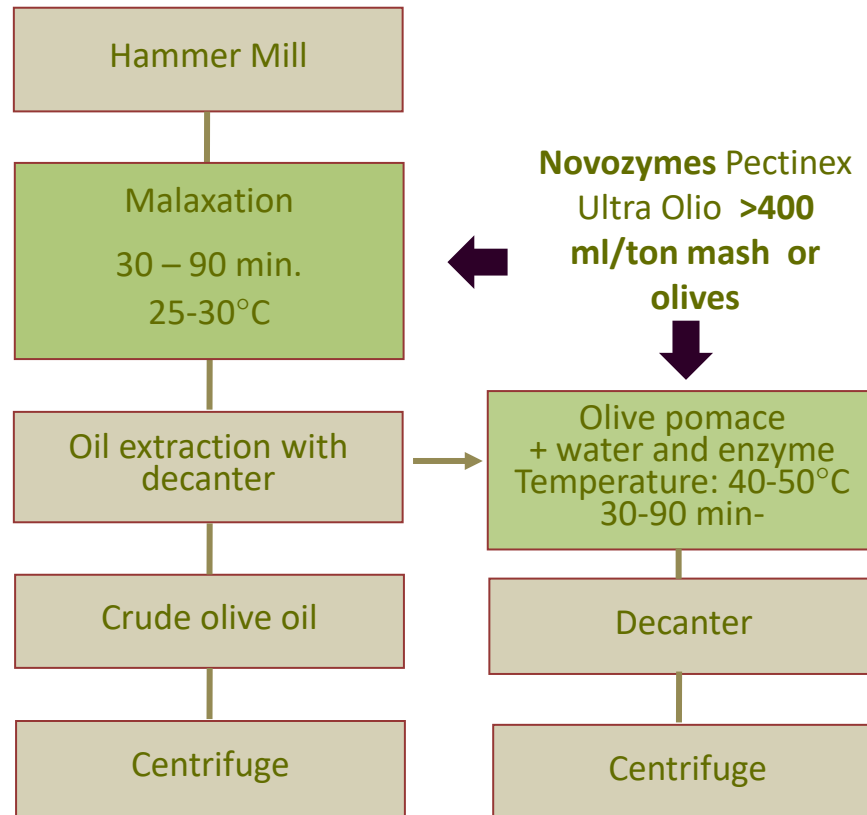
- In **continuous** lines, the solution can be added either directly into the mill or into the first compartment of the malaxing unit. In this situation, the use of a simple dosing pump is recommended.

- The initial dosage on early season and immature fruit can be 300-350 ppm to get maximum oil recovery. Dosage can be optimized during the season taking into account increased maturity of the fruit, residual oil content in the pomace and pumping capacity".

Olive oil production with fresh green olives

Clear and stable olive oil production with Novozymes Pectinex® Ultra Olio and olives with maturity index < 3.5

Option:
Addition of talc,
Super fine micro:
0.3-0.7 %
Fine micro: 1 - 4 %



- In **discontinuous** lines, a diluted enzyme solution - 10% in cold, clean tap water - is added directly into the mill and divided into 3-4 portions.
- In **continuous** lines, the solution can be added either directly into the mill or into the first compartment of the malaxing unit. In this situation, the use of a simple dosing pump is recommended
- The initial dosage on early season and immature fruit can be 300-350 ppm to get maximum oil recovery. Dosage can be optimized during the season taking into account increased maturity of the fruit, residual oil content in the pomace and pumping capacity”..

Recommendations to use Pectinex Ultra Olio

Novozymes Pectinex Ultra Olio	Target conditions
Retention time	45 mins.
Temperature	25-30°C
Olive moisture	56 %
Dosage /ton	100-600 ml

Features and benefits	
Enzyme effect (minimum)	10 mins.
Minimal time to release oil	>30 mins.
Achieve full effect of Pectinex Ultra Olio	45-60 mins.



Pectinex Ultra Olio Product Features

- Pectinex Ultra Olio contains
 - **Strong pectinase activities in terms of robust pH, temperature variations and tannin flexibility and also some very**
 - **Specific and advantageous side activities (pectinases, e.g. arabinanases, hemicellulases, e.g. mannanases and cellulases)**
 - **Especially is it strong in Rhamnogalacturanase, which has been proved to be one of the crucial key activities for efficient solid/liquid separation**
- Pectinex Ultra Olio contains several enzymes activities, which reacts well in a pH range between 3.5 and 5.5.
- Pectinex Ultra Olio works in range of 10-60°C with optimum temperature 25-50 °C
- Pectinex Ultra Olio fulfill highest quality and Food standards



Boost yields and get the most out of your olives

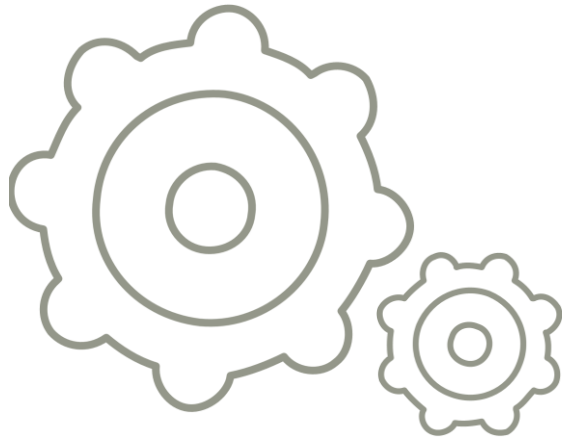
	Control	200	300	400
Enzyme dosage ml/MT				
Oil in fruit (%)	12.66	13.2	11.28	11.18
Moisture in fruit (%)	65.51	65.57	65.27	64.81
Maturity index	1.14	1.27	1.23	1.17
Oil in dry (%)	36.71	38.34	32.48	31.77
Crusher grid (mm)	5	5	5	5
Malaxing time (min)	73.5	67.5	69	69.5
Malaxing temperature (°C)	27.1	29	30.4	28.3
Processing speed (Kg/h)	7000	7000	7000	7000
Total fruit processed (Kg)	5000	5000	5000	5000
Total oil obtained (Kg)	394.4	453.4	418.3	444.3
Industrial Efficiency (%)	62.3	68.7	74.2	79.5
Oil recovered (%)	7.89	9.07	8.37	8.89
Oil in pomace (%)	4.77	4.13	2.91	2.29
Diff. loss vs control (%)	0	0.64	1.86	2.48
Oil Recovery per ton (kg)	0	6.4	18.6	24.8
Gross benefit per ton (US\$)	0	\$19	\$56	\$74
Enzyme cost per ton (US\$)	0	\$9	\$14	\$18
Net benefit per ton (US\$)	0	\$10	\$42	\$56

Boost your
milling yields

1-2%
Higher oil
yields/tons









Pectinex Ultra Olio

Smother operations, whatever your olive type



- **Faster, more efficient oil and water separation**
- **Quicker, better oil clarification**
- **Works with all extraction systems**
- **Better oil stability**









Arbequina firmness according to Maturity index

Maturity Index	0	1	2	3	4	5	6	7
Pictures of Maturity Index								
Firmness data (Kgf/cm ²)	Data#1	Data#2	Data#3	Data#4	Data#5	Data#6	Data#7	Data#8
Extractibility (% w/w)	Data#1	Data#2	Data#3	Data#4	Data#5	Data#6	Data#7	Data#8
Industrial Efficiency (IE %)	Data#1	Data#2	Data#3	Data#4	Data#5	Data#6	Data#7	Data#8

Arbequina firmness according to M.I



Enzyme recommendation according to fruit firmness

Maturity Index	0	1	2	3	4	5	6	7
Pictures of Maturity Index								
Picual	Light Green				Orange	Red		
Arbequina	Light Green			Orange	Red			
Hojiblanca	Light Green		Orange	Red				

Pectinex Ultra Olio Product Features

Pectinex Ultra Olio contains

Strong pectinase activities in terms of robust pH, temperature variations and tannin flexibility and also some very

Specific and advantageous side activities (pectinases, e.g. arabinanases, hemicellulases, e.g. mannanases and cellulases)

Especially is it strong in Rhamnoglacturanase, which has been proved to be one of the crucial key activities for efficient solid/liquid separation

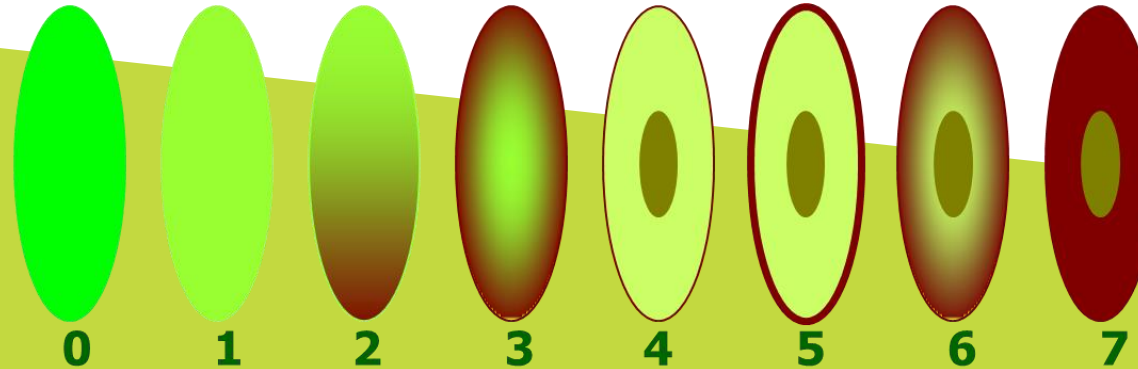
Pectinex Ultra Olio contains several enzymes activities, which reacts well in a pH range between 3.5 and 5.5.

Pectinex Ultra Olio can work in a range of 10-60°C with optimum temperature 25-50 °C

Pectinex Ultra Olio fulfill highest quality and Food standards

Pectinex Ultra Olio Dose adjustment

Olive
Maturity
Index



Olive Varieties (extraction difficulty)

Medium: Barnea, Arbiquena, Picholine, Coratina

Difficult: Manzanillo, Picual, Hojiblanca, Leccino

Crushing grid size

The smaller the higher the enzyme dose

Can vary from single to doble

Processs equipment

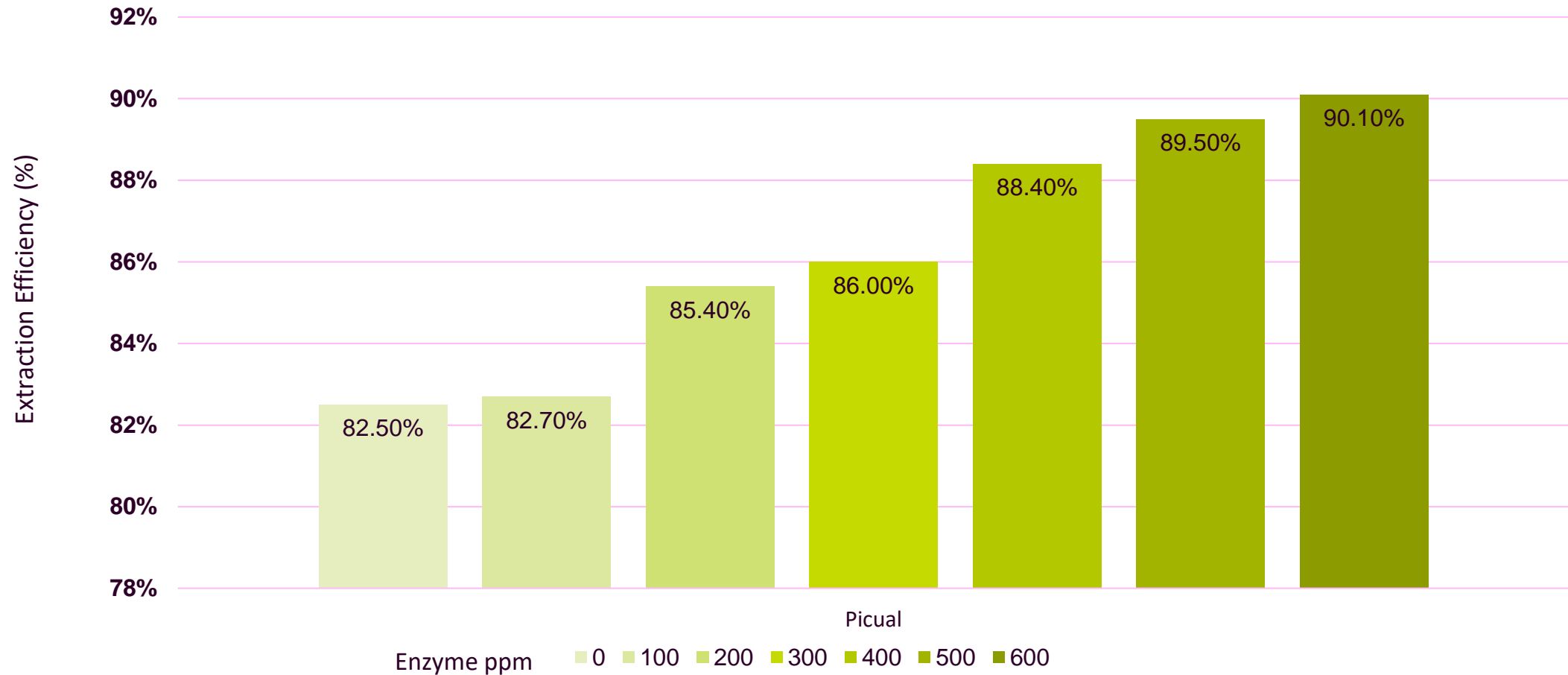
2 steps *versus* 3 steps

Decanter



Olive Oil Extraction efficiency (1) - dose response Pectinex Ultra Olio

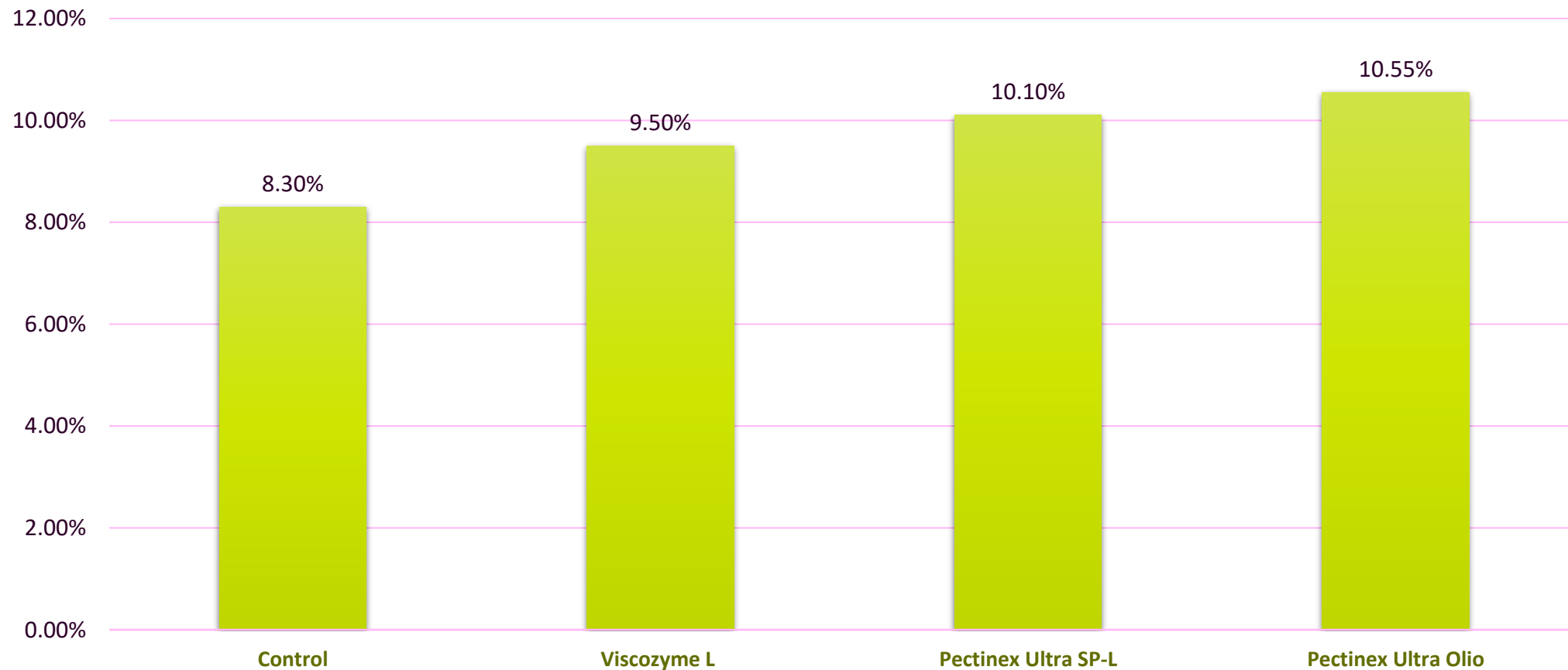
Impact olive oil extraction efficiency Picual olives 58.8% moisture



Olive Oil Extraction efficiency (2)

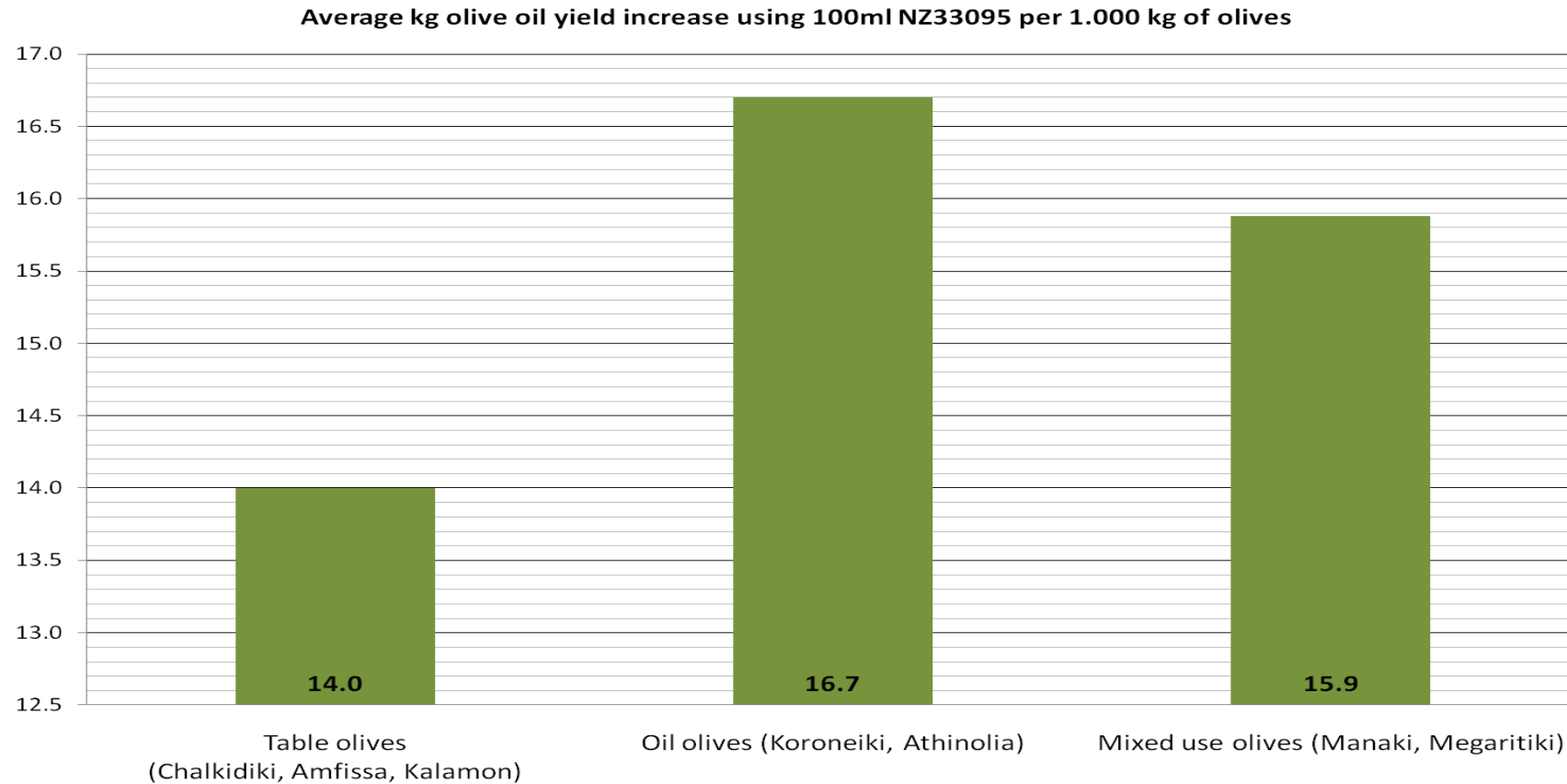
- Different enzyme Response

Trial with several Novozymes formulation dose at 300 ppm with Arbequina olives at maturity index 1.5

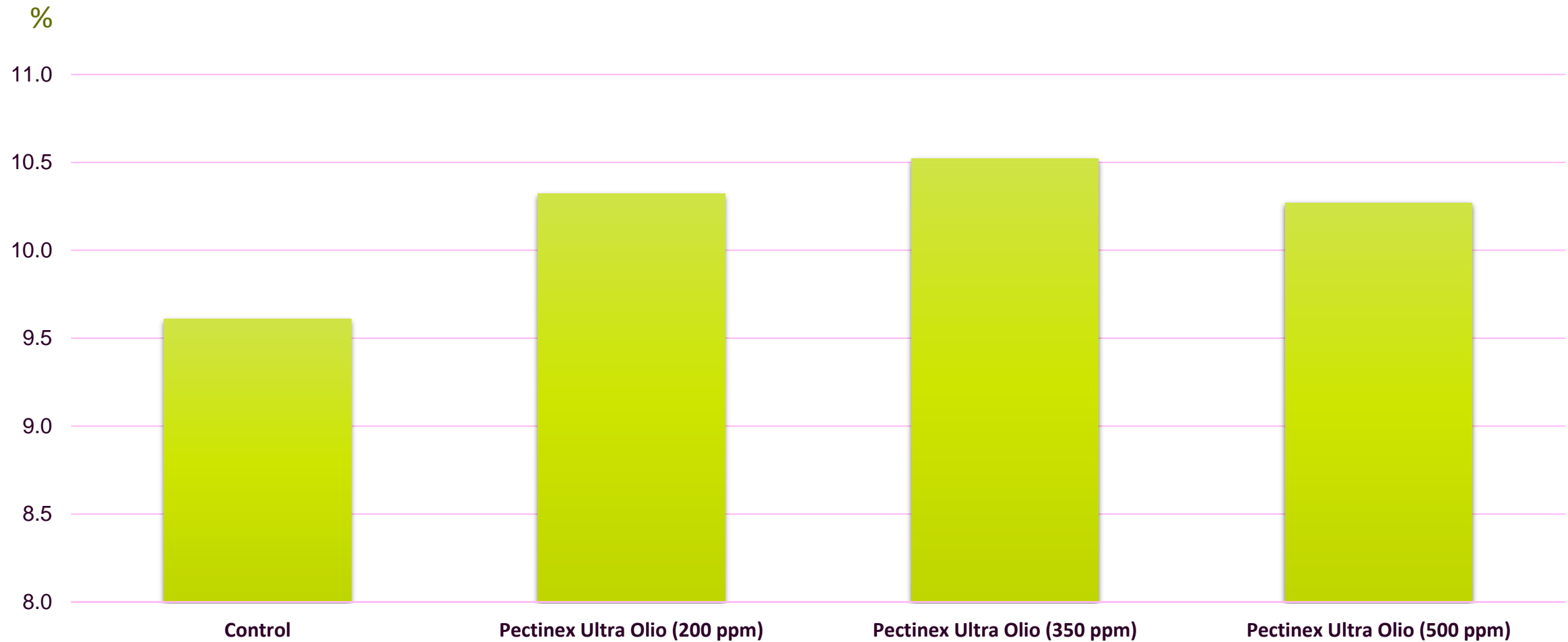


Pectinex Ultra Olio results (3) from Greece

Average results obtained with different olive varieties at 100 ppm with NZ 33095, precursor enzyme for Pectinex Ultra olio



Arbequina – Oil Yield %

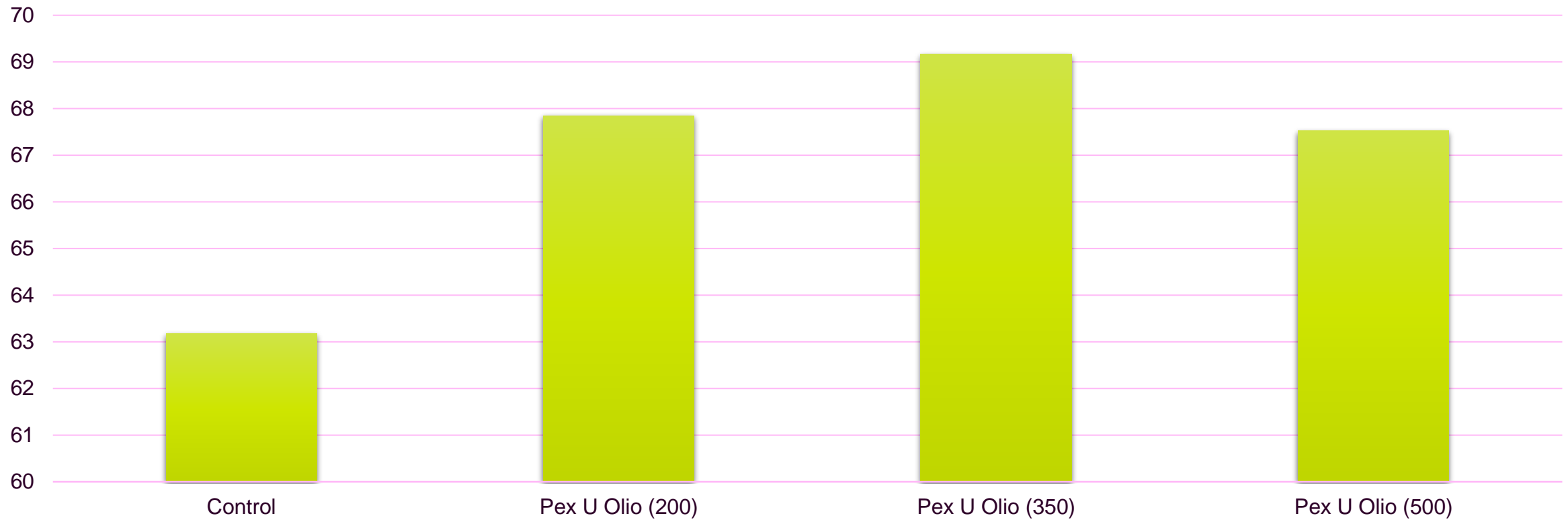


Abencor lab trial - Arbequina maturity index 0.67

Arbequina – Industrial Efficiency %



Arbequina - Industrial Efficiency (%)



Picual – Oil Yield %



Picual – Industrial Yield %



Picual - Industrial Efficiency %



Analytical Parameters



TABLE XXIII. 'PICUAL' R. I. 0.73. ACIDITY.
(% OLEIC ACID)

ACIDITY			
Control	0.13		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	0.14	0.14	0.15

TABLE XXIV. 'PICUAL' R. I. 0.73. PEROXIDE INDEX
(MEQ. O₂/KG)

PEROXIDE INDEX			
Control	3.75		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	3.51	4.28	4.30

TABLE XXV. 'PICUAL' R. I. 0.73. K₂₇₀

K ₂₇₀			
Control	0.11		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	0.10	0.10	0.10

TABLE XXVI. 'PICUAL' R. I. 0.73. K₂₃₂

K ₂₃₂			
Control	1.47		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	1.74	1.08	1.17

TABLE XXVII. 'PICUAL' R. I. 0.73. DELTA K

DELTA K			
Control	0		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	0	0	0

TABLE XXVIII. 'PICUAL' R. I. 0.73. STABILITY (H)

STABILITY			
Control	15.6		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	15.4	15.6	15.8

TABLE XXIX. 'PICUAL' R. I. 0.73. COLOR DIFFERENCE

COLOR DIFFERENCE			
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	0.41	3.13	4.65

TABLE XXX. 'PICUAL' R. I. 0.73. ORGANOLEPTIC SCORE

ORGANOLEPTIC SCORE			
Control	7.6		
	DOSAGE (ML/T)		
ENZYMATIC COMPLEX	200	350	500
PECTINEX®	7.8	7.5	7.7

Instituto de la Grasa, Sevilla, Trials Results – Conclusions (1)

Status 2016 season

- In all cases the **texture and the extraction** behaviour of the **olive paste slightly improved** compared to control.
- For both varieties studied, the total volume obtained increases when all dosages are used, being in ‘Arbequina’ variety the highest phases separation when 500 ml/t is used and in ‘Picual’ variety when 200 ml/t is added, this can probably occur due to the initial moisture content in the raw material used.
- In ‘**Arbequina**’, the best oil yields occurs when using 350 ml/t, that means an increase of 0.91 points (9.47%).
- In ‘**Picual**’, the best oil yields occurs when using 500 ml/t, that means an increase of 0.82 points (9.27%).
- In the following oil quality parameters: acidity, K270, K232, delta K, organoleptic score, important differences between control and treated samples, **were not found**.
- In ‘Arbequina’ variety, for oil parameters such as stability, a slight increase, compare to the control, occurs when all dosages are used, not important differences between control and treated samples were found in Picual ‘variety’.



Instituto de la Grasa, Sevilla, Trials Results – Conclusions (2)

Status 2016 season

- *In 'Picual' variety, it is observed a slight peroxide index trend to increase compared to the control oil when 350 and 500 ml/t dosages are used and a slight dosage effect for color difference parameter, for these parameters not important differences between control and treated samples were found in 'Arbequina' variety.*

Outcome of Enzymes usage in OO processing

- We have shown with use of enzymes in olive oil processing to achieve **yield increase** and **capacity increase** without changing final olive oil qualities
- Olive oil produced with use of enzymes achieved in world wide olive oil competitions highest rankings in their classes.
- Enzymes are natural flexible tools in olive oil processing to improve significantly **performances to produce olive oil and to overcome shortages of olive resources, due to e.g. diseases, climate changes, etc.**
- Use of enzymes in pomace of olive can help to **extract naturally antioxidants and other healthy substances**
- Use of enzymes allow a more clean olive oil production, **cleaner equipment with less energy intake, less and dryer waste, all in all, more sustainable.**



Questions & Answers (1)

Q: *Is Pectinex Ultra Olio GMO derived enzyme ?*

A: Pectinex Ultra Olio is produced by the classical method, meaning that the production strain is NOT genetically modified

Q: *Can it be used for organic/biological olive oil production?*

A: Yes, due to the fact that production strain has NOT been modified genetically, it is suitable for organic production.

Q: *From which Aspergillus is Pectinex Ultra Olio produced?*

A: Pectinex Ultra Olio is produced from *Aspergillus aculeatus* and *Aspergillus niger*. Review the PDS, where both strains are mentioned. If asked, if it is from *A. Aculeatus*, we can answer YES.

Questions & Answers (2)

Q: Is it legal to use the enzyme for olive oil?

There is “no clear answer” related to countries, e.g. for virgin olive oils under European Regulation EC No 1234/2007 regulates olive oils in Article 118 (p.110-11) and Annex XVI (p. 82-282) specifies there is no clear yes or no, for the use of enzymes in olive oils. **In australia and other new olive oil processing countries, use of enzymes is allowed also for virgin oil**

So far, for the production of “virgin olive oils”, the use of processing aids, such as enzymes and also talc “should be avoided”. Therefore enzymes and talcs are not forbidden.

***Cold pressed virgin oils, as part of virgin oil production**, is probably the exception with no use of enzymes (but <5 % market segments)

For all other oils,, the use of food grade enzymes is permitted

6 categories of Olive Oil in - EU regulation Enzymes

- | | |
|-----------------------------|-----------------------------|
| 1. Virgin olive oil* | cold pressed virgin oil, No |
| 2. Refined olive oil | OK |
| 3. Olive oil | OK |
| 4. Crude olive pomace oil | OK |
| 5. Refined olive pomace oil | OK |
| 6. Olive pomace oil | OK |

Questions & Answers (3)

In **Spain**, the use of enzymes (carbohydrases derived from *Aspergillus aculeatus*) are still allowed for the production of all olive oils. In 1986, the positive list for additives and processing aids was established. Later in 1989, this list was modified to include enzymes as processing aids under 2.2. (See: “Orden de 30 noviembre 1989 (BOE del 9 diciembre 1989)”

The new EC Regulation 1234 from 2007 should overrule the Spanish regulation, according to Novozymes’ Regulatory affairs interpretation.

With upcoming FIAP Regulation for food enzymes can be a review to permit use of enzymes in EU countries ?

Questions & Answers (4)

Q: Does the olive oil need to be labeled, when an enzyme is used during processing?

A: NO. Enzymes are processing aids, so there is no need to declare their use on a label on the finished product.

Q: Will the olive oil quality be affected or changed, when I use the Novozymes enzyme?

A: NO. The oil quality and taste do not change.

Q: Can the enzyme be detected in the final oil, when I have used it in the process?

NO. Pectinex Ultra Olio cannot be detected in the final oil. The enzyme is **water soluble** and is removed with the water phase and/or with the pomace.

Pectinex ultra Olio does not contain any preservatives (no benzoate, no sorbate).

Questions & Answers (5)

Q: What is the difference between Pectinex Ultra Olio and Novozymes' older product, – Novozymes Olivex®, that was sold in the past?

A:

Pectinex Ultra Olio is a further development and a more complete formulation, which ensures good performance under varying conditions of the olive fruit, including maturity and cell structure.

The enzyme activities within Olivex also remain in Pectinex Ultra Olio .

Questions & Answers (6)

Q: How much yield increase can I expect?

A:

The achieved yield increase depends on the olive variety, maturity stage, moisture content and processing conditions.

The average yield increase can be approx. 1-2 % or >10 liter more oil per ton of olives.

Several cases demonstrating an increase of up to 20 kg, under optimal conditions and processes.

One of the key benefits of the enzyme is that you can process the olives at an **early maturity** stage and still achieve high oil yields.

Questions & Answers (7)

Q: Can Pectinex Ultra Olio eliminate the use of talc?

A:

This is a good and interesting question. We have seen, that in several cases the best results were demonstrated with combinations of talc and enzymes, especially when the olives have a very high moisture content, >55%, or with difficult varieties, Picual, Manzanilla, Hojiblanca, Leccino. In these cases, talc helped to improve compaction.

In other cases, talc could be eliminated or demonstrated no benefits.

Questions & Answers (8)

Q: Can Pectinex Ultra Olio be considered as processing aids?

A:

The definition of processing aids for enzymes is defined with no presence in final product and playing no active role in final product. Enzymes in olive oil processing, as mentioned, can not be present in final olive oil and is also not playing an active role in final products.

Therefore enzymes are processing aids in olive oil processing.

Q: Can Pectinex Ultra Olio be used for USA imported olive oil?

A:

Yes. For the USA it is mandatory, that the strains are permitted. Both strains from Pectinex Ultra Olio are considered as GRAS (generally recognized as safe) and enzymes can be therefore used for Olive oil imported into USA.

Summary with use of Pectinex ultra olive

We have shown with use of enzymes in olive oil processing to achieve yield increase and capacity increase without changing final olive oil qualities

Olive oil produced with use of enzymes achieved in world wide olive oil competitions highest rankings in their classes.

Enzymes and optional talc are natural flexible tools in olive oil processing to improve significantly performances to produce olive oil and to overcome shortages of olive resources, due to e.g. diseases, climate changes, etc.

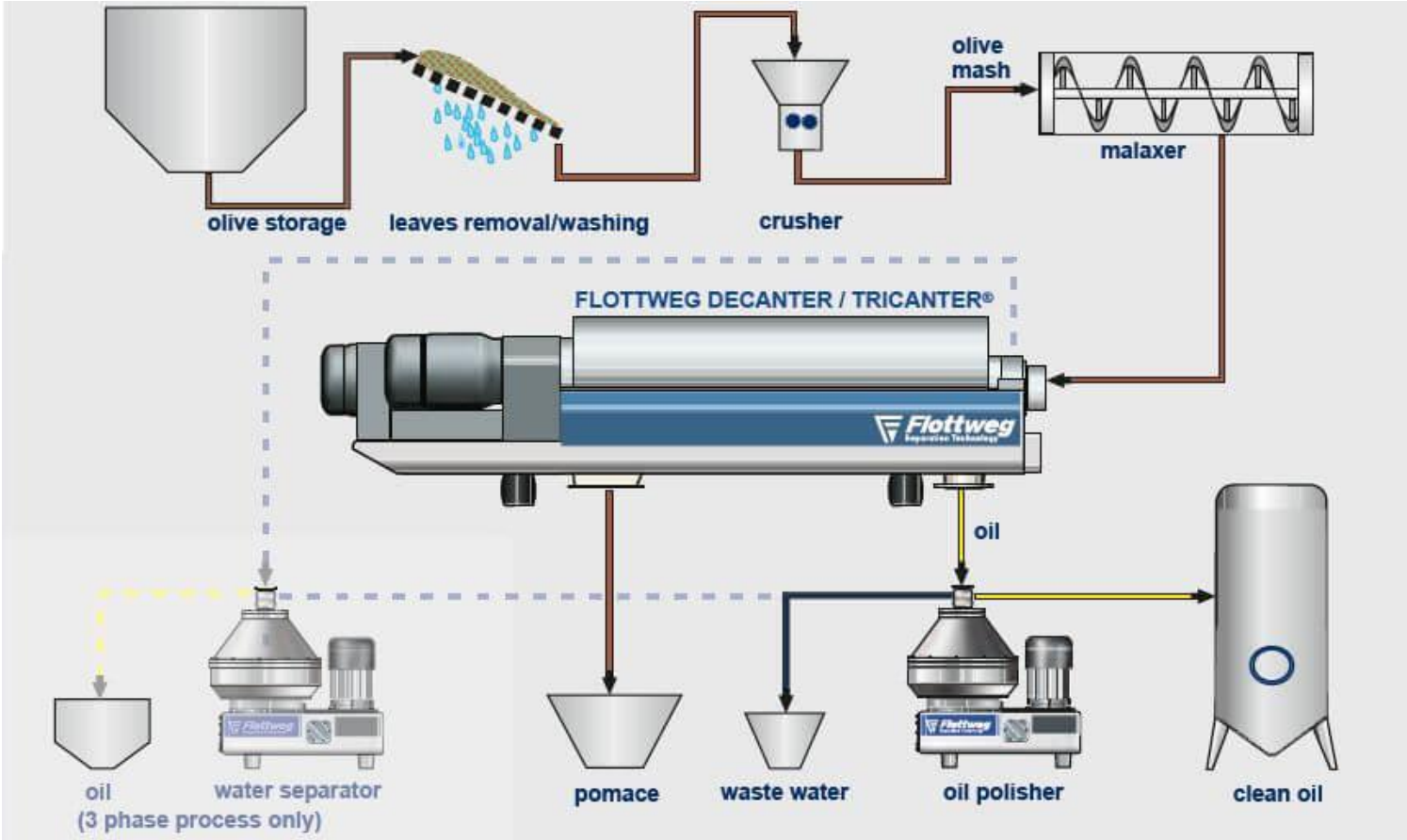
With use of enzymes in malaxer, we achieve much dryer pomace !

Use of enzymes in pomace of olive can help to extract naturally antioxidants and other healthy substances

Use of enzymes will allow a more clean olive oil production, more cleaner equipment with less energy intake, less and dryer waste, all in all, more sustainable.

Our oils & fats colleagues can assist, if acidity of olive oils needs to be reduced, please contact us

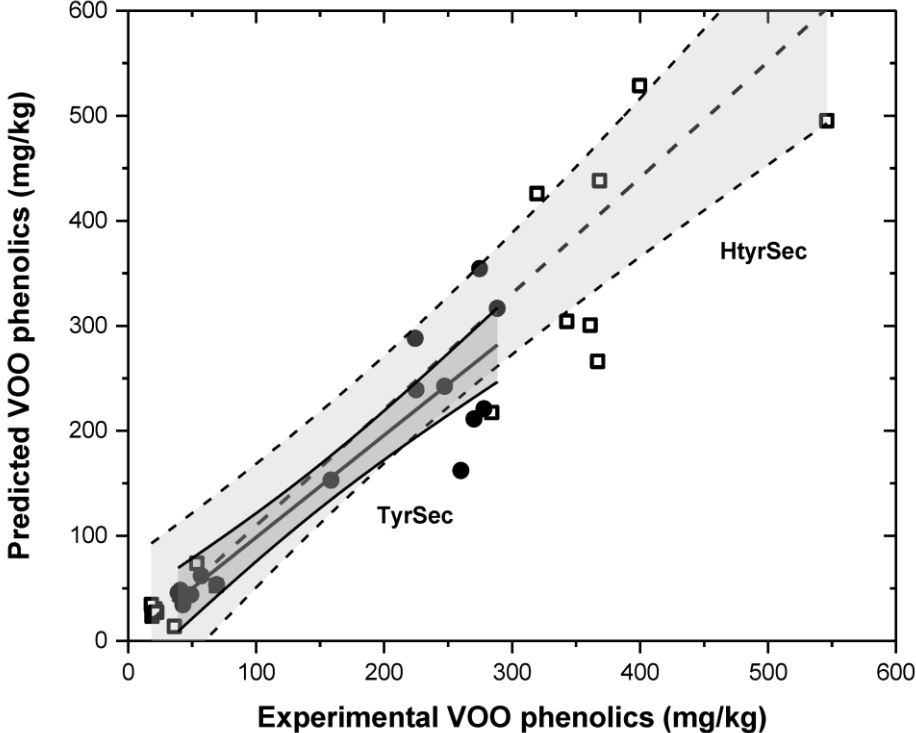
Legend (1)



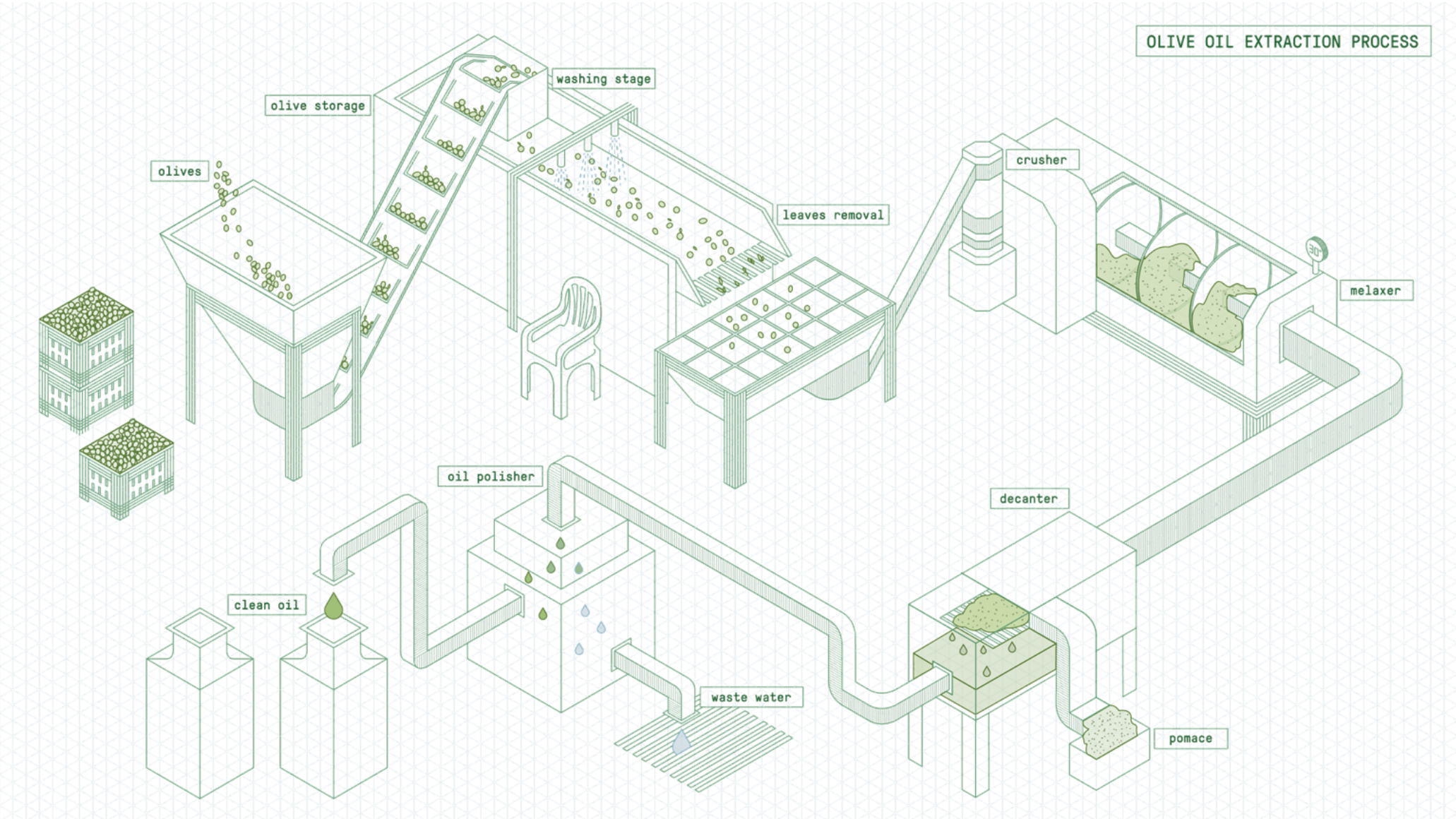
Legend (2)



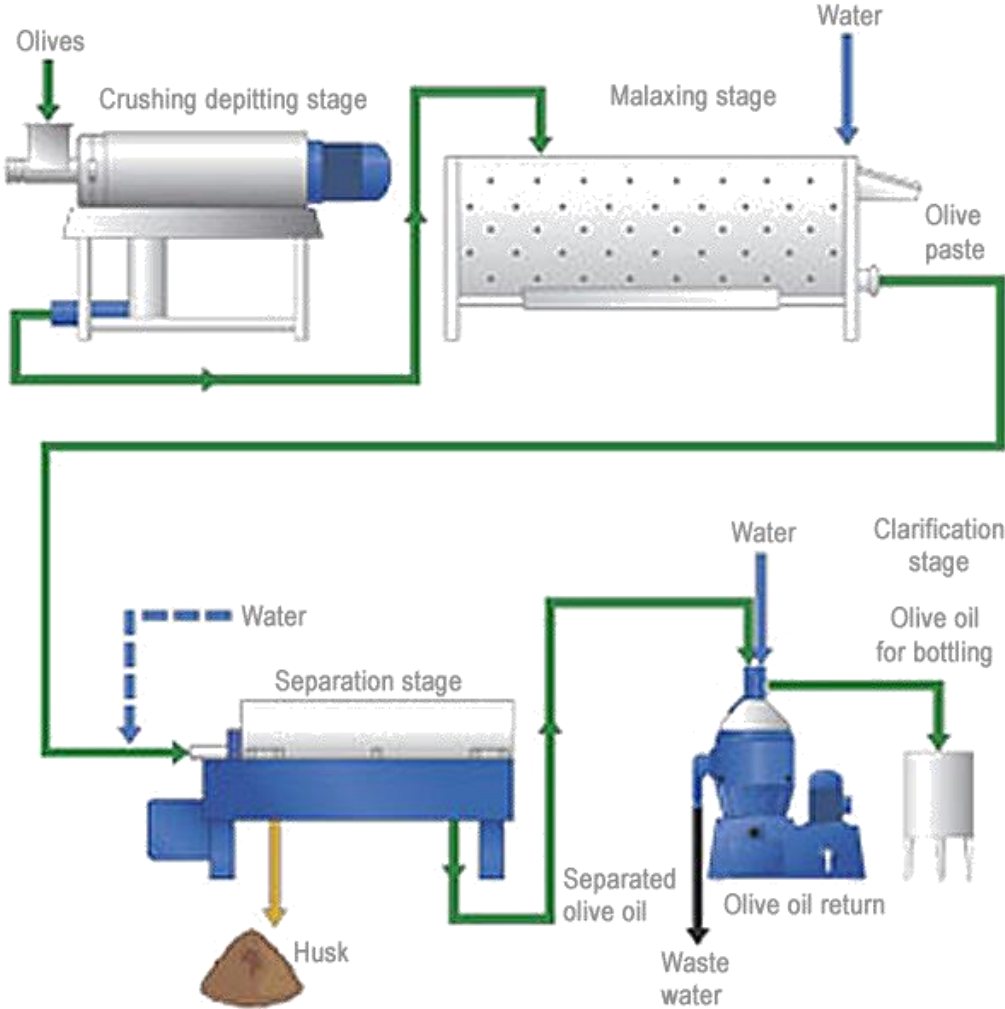
Legend (3)



OLIVE OIL EXTRACTION PROCESS



Legend (4)



novozymes[®]



Rethink Tomorrow