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Chlorophyll Reduction in Rape Seeds and its Influence on the Bleaching Earth Consumption during Refining

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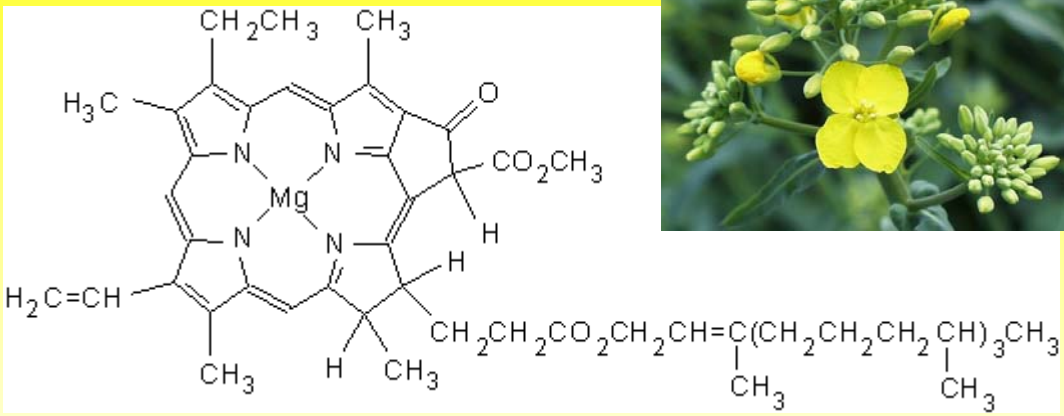
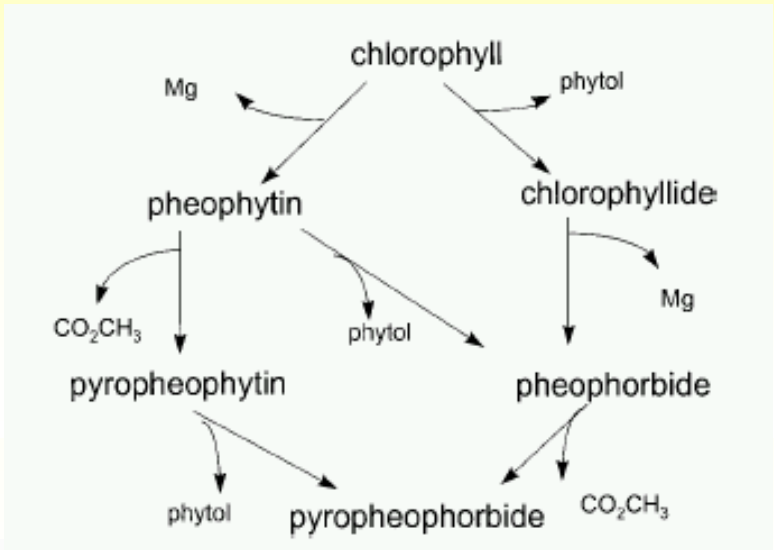
Outline

- **Influence of chlorophyll on rape seed oil quality**
- **Effect of the chlorophyll content in crude rapeseed oils on bleaching earth consumption**
- **Chlorophyll reduction strategies**
- **Conclusions**

Chlorophyll in Rapeseed Oil





Chlorophyll

- is essential for the photosynthesis
- occurs in all edible oils
- reduces the oxidative stability, taste and shelf life

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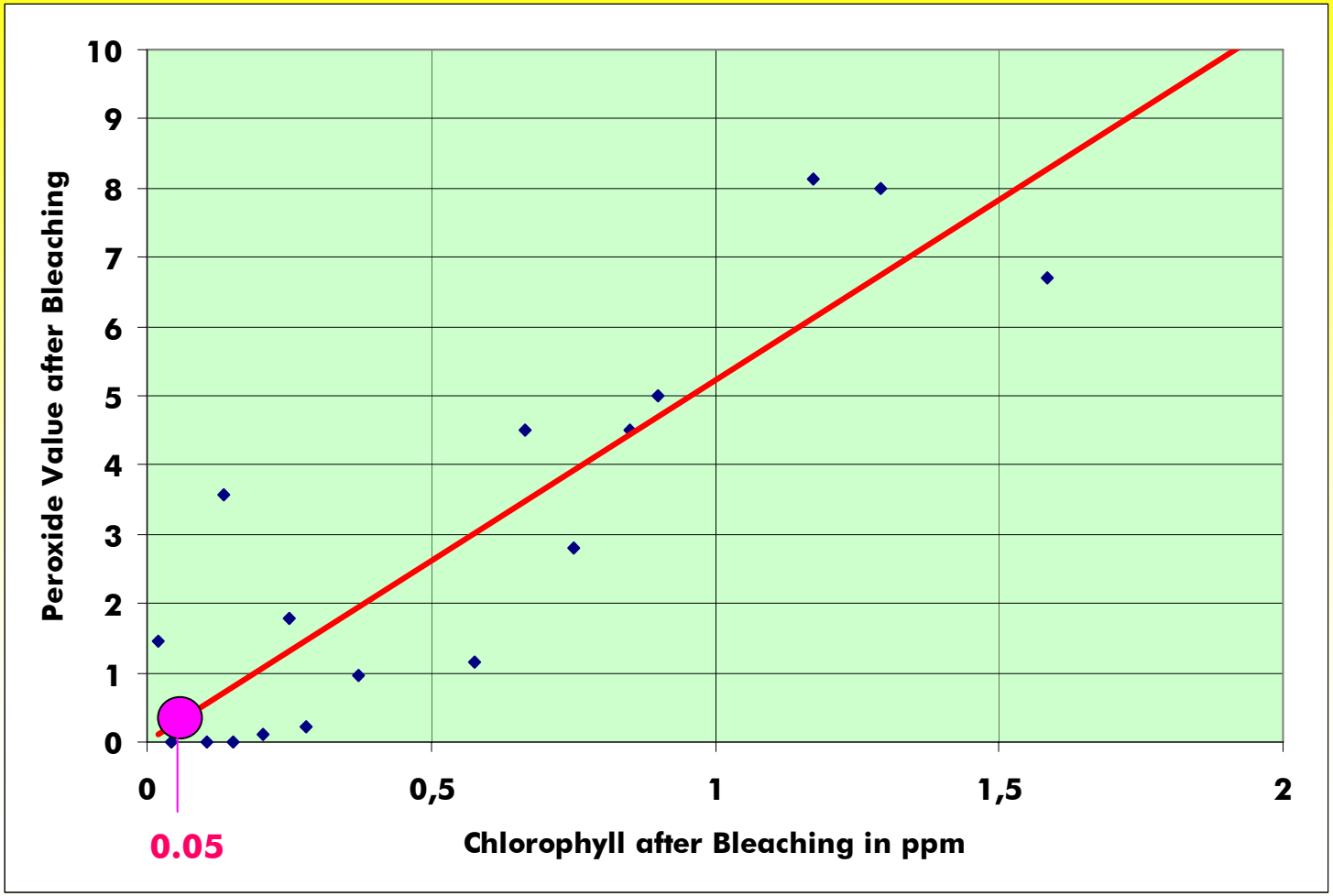
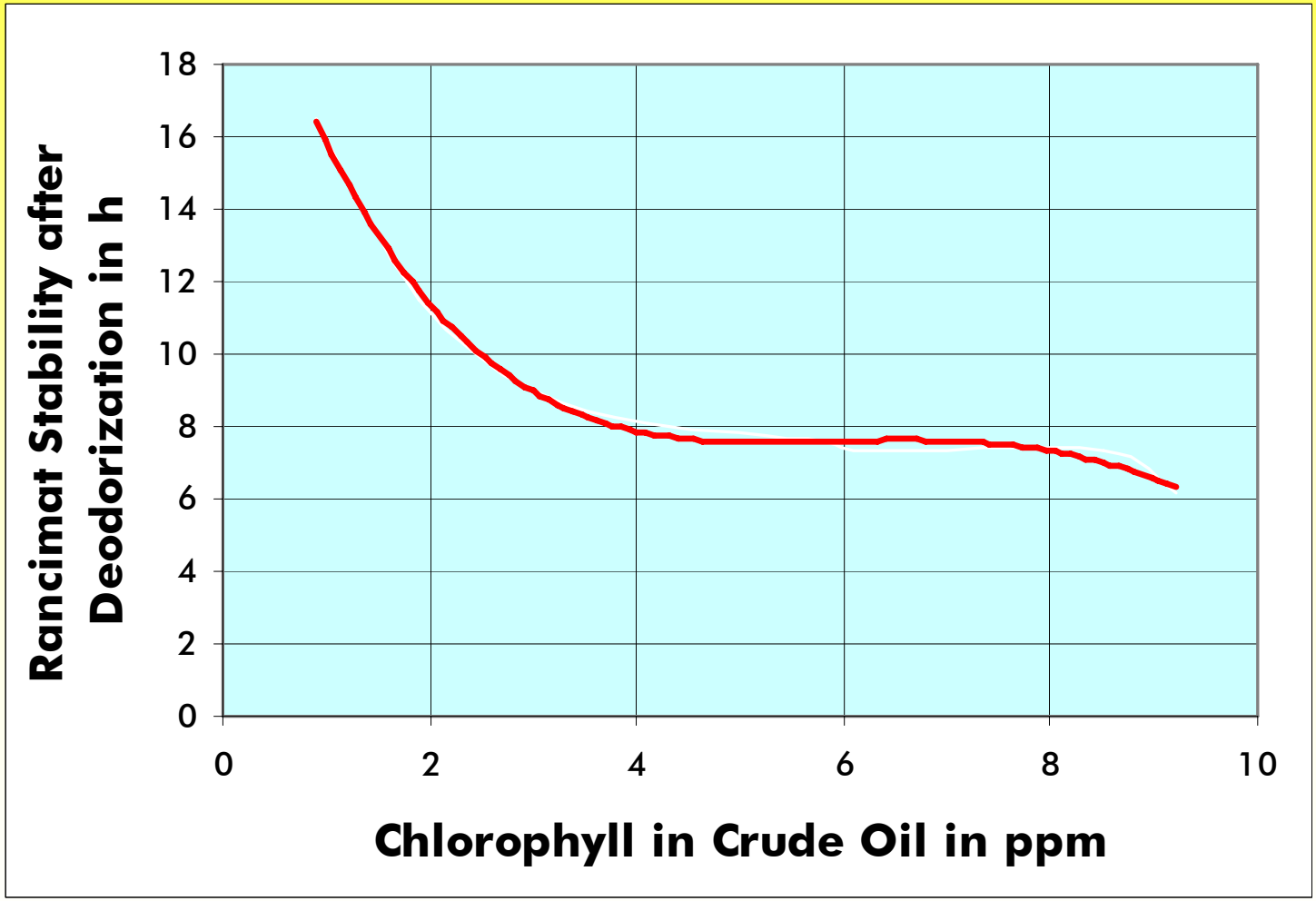
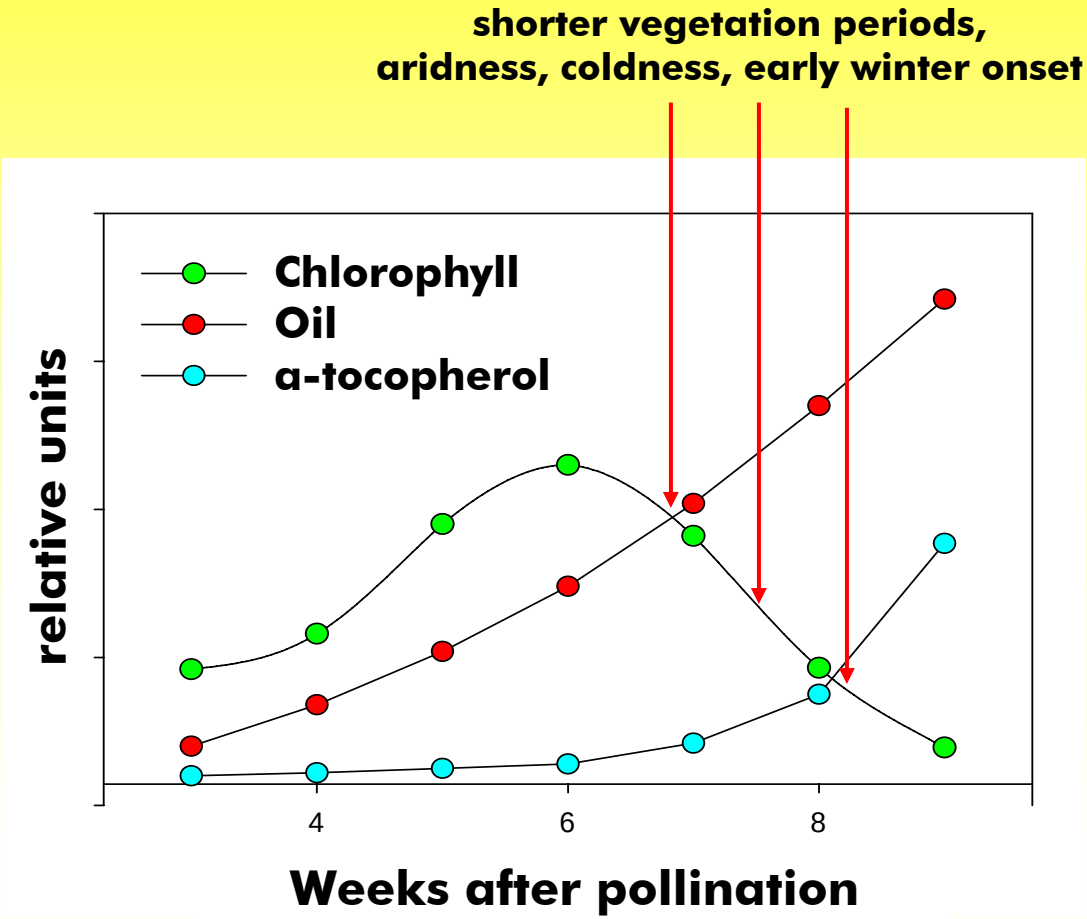
Chlorophyll and Peroxide Value



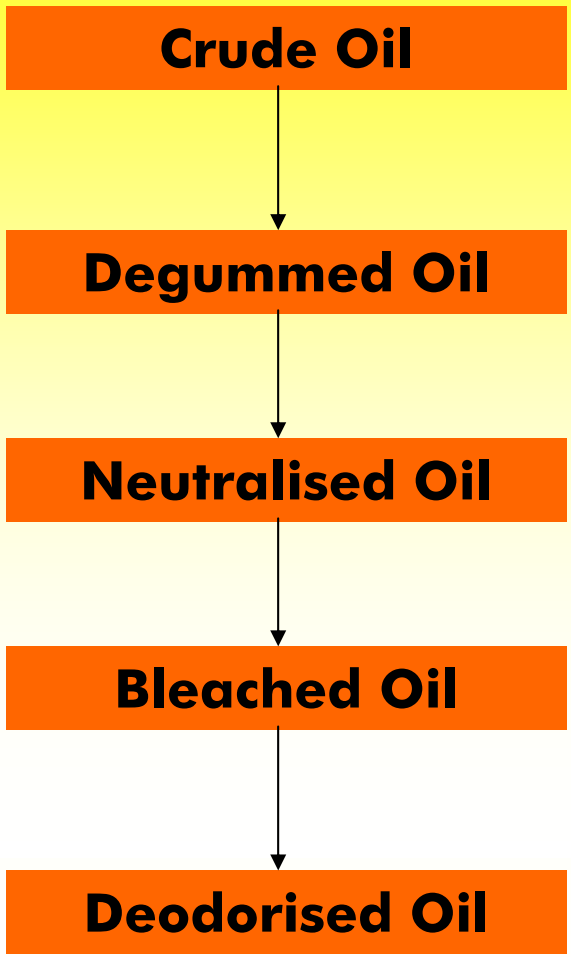
Chlorophyll and Oxidation Stability

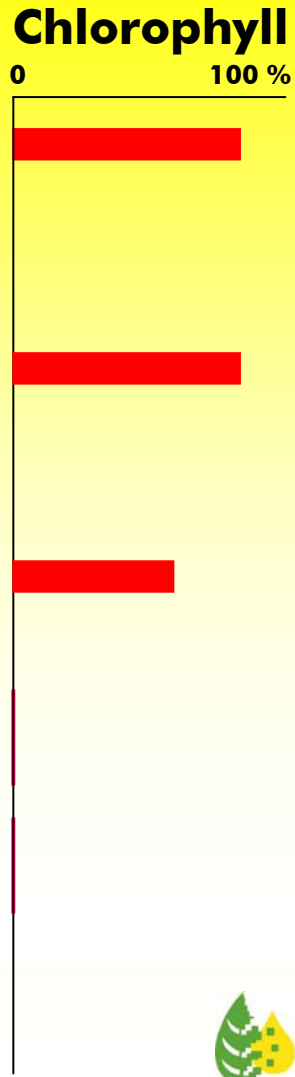


The Rapeseed Problem



Chlorophyll Removing by Chemical Refining





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Economic Data for Bleaching

- **Rapeseed production, worldwide*: 49 Mio. t/a**
- **Rapeseed oil production, worldwide*: 18 Mio. t/a**
- **Bleaching earth consumption**: ~ 0,11 Mio. t/a**
- **Reduction of 0,1% bleaching earth consumption leads to**
  - **16.000 t/a less bleaching earth (consumption and disposal)**
  - **4.800 t/a less oil losses**
  - **preservation of minor components (tocopherols, sterols)**

Which amount can be saved only by reducing the chlorophyll content in the crude oil (resp. rapeseed) ?

*** DUSSER, 2007; ** 90% refining, 0,7% bleaching earth**

Test Scheme of Laboratory Experiments

Chlorophyll a

Refined rapeseed oil

→ **Degummed, (neutralised) rapeseed oil**

Chlorophyll: **2,065 ppm**
3,73 ppm
5,75 ppm
8,08 ppm
20,9 ppm

Parameters:

- **Bleaching earth consumption**
- **Temperature**
- **Bleaching time**
- **Citric acid concentration**

↓ **Bleaching**

↓ **Deodorisation**

↓ **Analysis**

	Oil 1 degummed, neutralised	Oil 2 degummed, neutralised	Oil 3 degummed
Peroxide Value	23,20	4,15	1,00
Anisidine Value	-	6,87	-
Acid Value	0,13	0,13	1,27
UV–Extinction at 232 nm	-	1,81	-
Water	-	0,07 %	-
Carotine	-	54,7 ppm	-
Chlorophyll a	3,73 ppm	3,50 ppm	4,39 ppm
Phosphorus	< 1 ppm	4,9 ppm	< 1 ppm
Calcium	-	1,21 ppm	-
Iron	-	0,082 ppm	-
Manganese	-	<0,01 ppm	-
Copper	-	<0,01 ppm	-
Magnesium	-	0,19 ppm	-
Total Tocopherol	-	768 ppm	-

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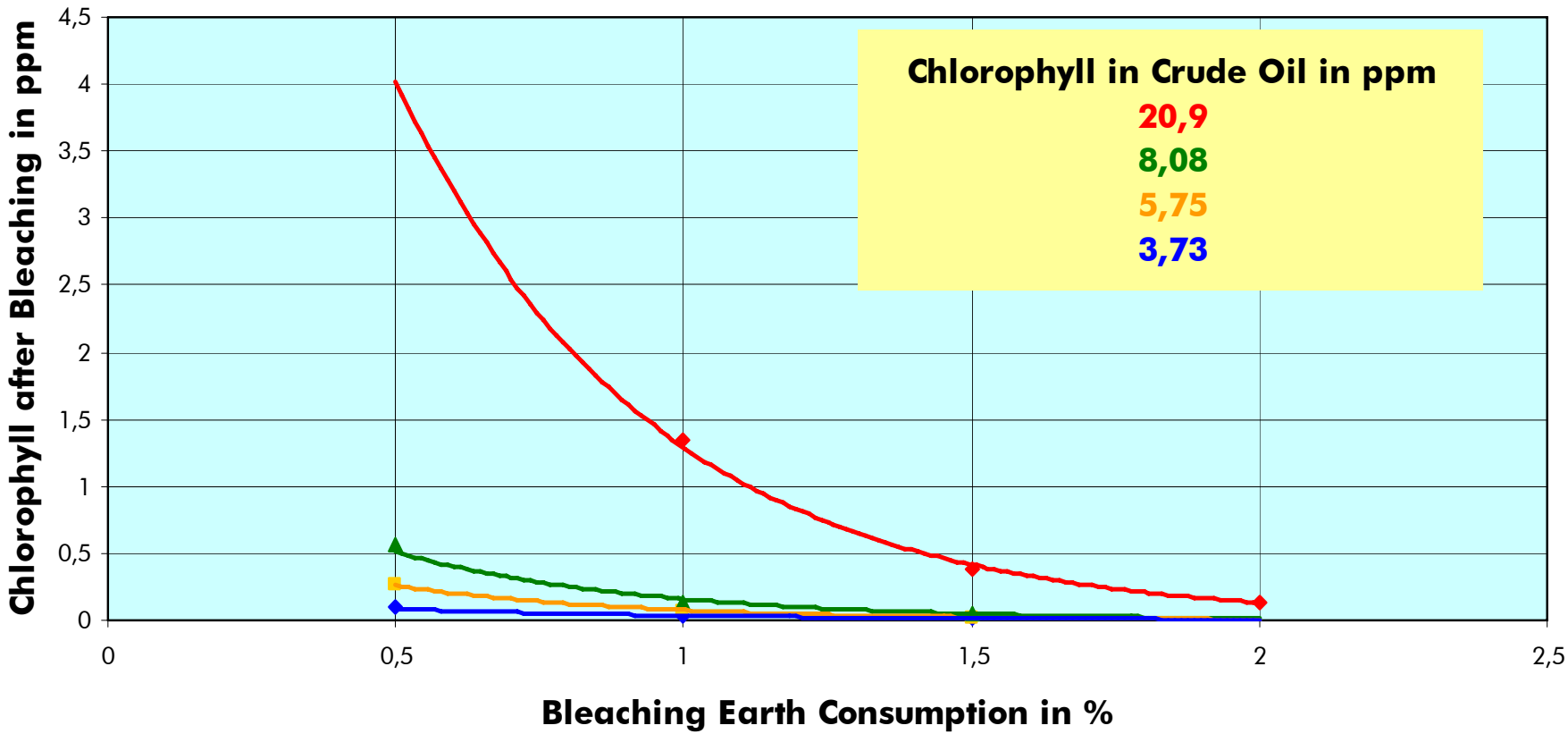
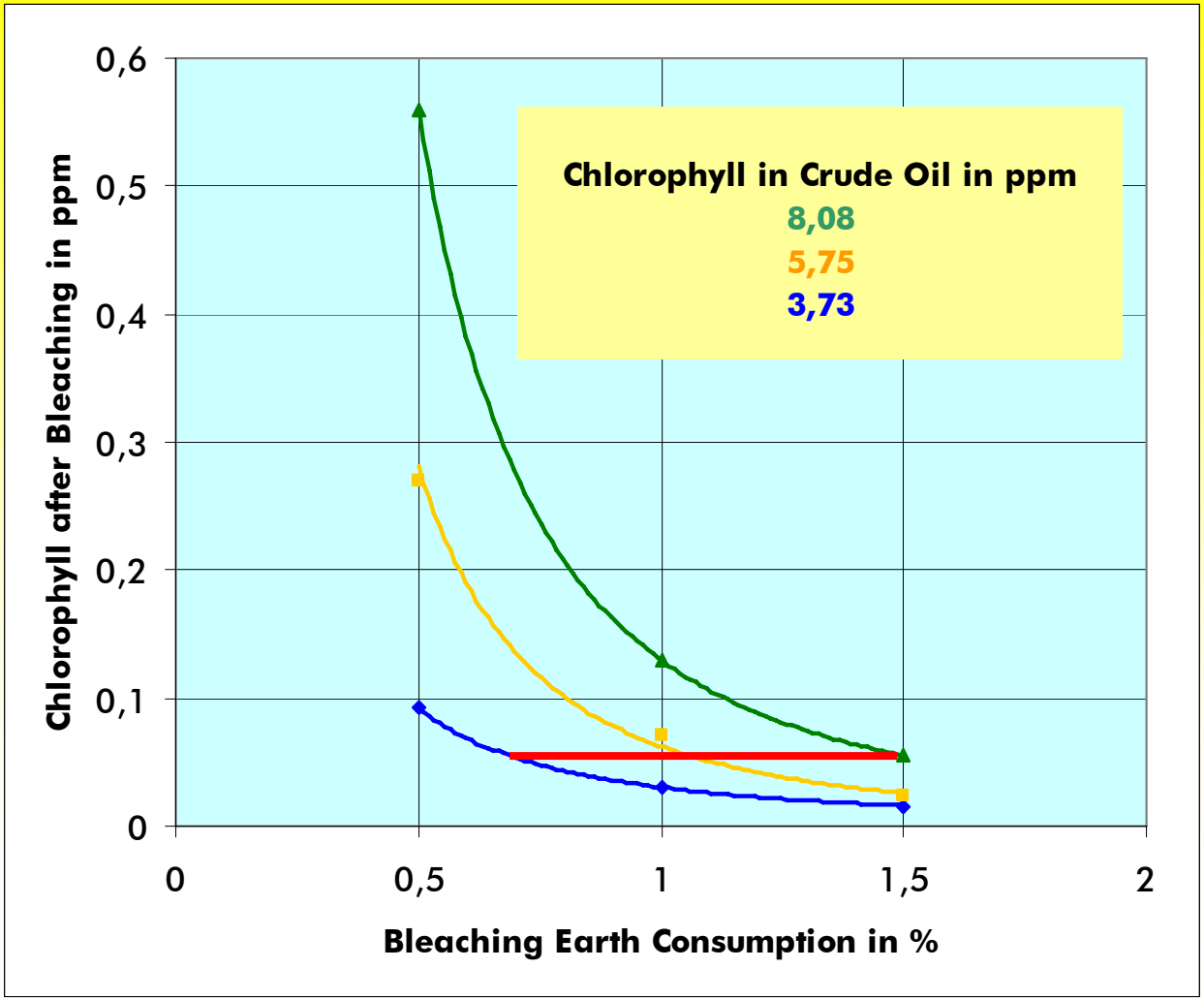
Bleaching Behaviour



Bleaching Earth Consumption



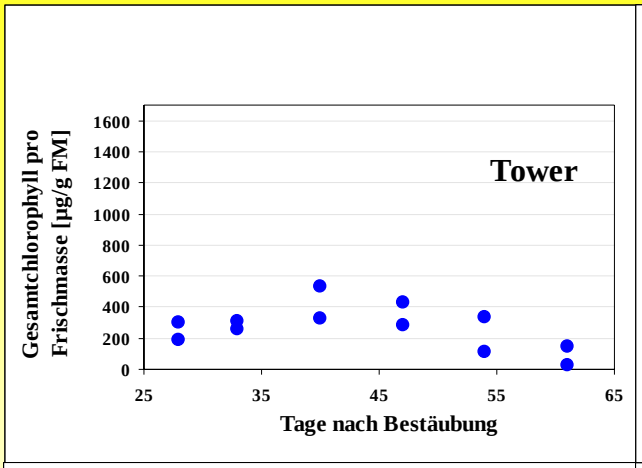
Not only very high chlorophyll contents in crude oils can be a problem.

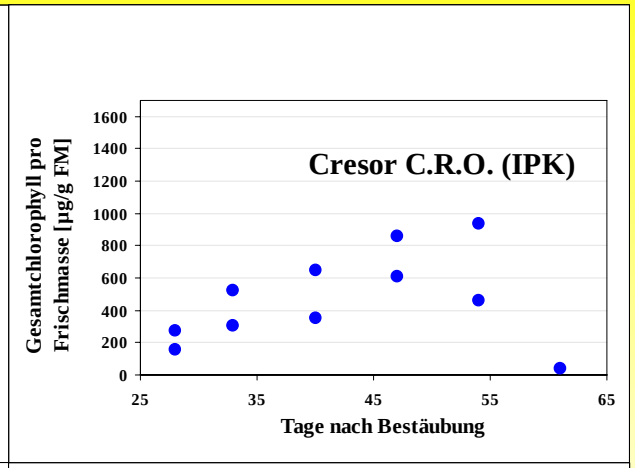
Low chlorophyll in crude oil leads to significant lower bleaching earth consumption !

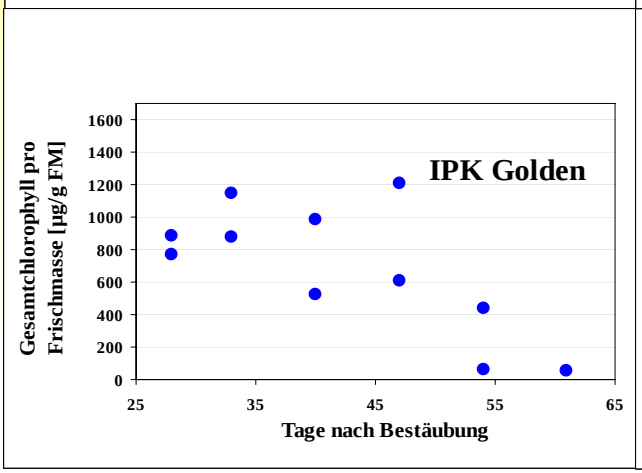
Chlorophyll Reduction Strategies

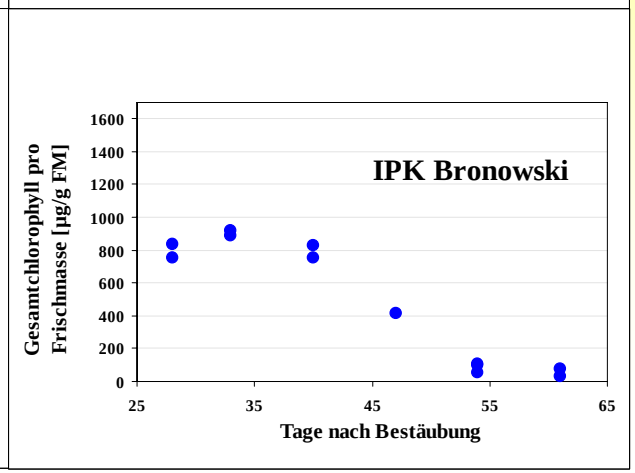
- **Classical plant breeding**
- **Transgenic Plants**
  - **Chlorophyll biosynthesis: Inactivation of chlorophyll producing enzymes**
  - **Chlorophyll catabolism: Overexpression of chlorophyll degrading enzymes**
- **Use of recombinant proteins to develop an enzymatic chlorophyll removal process**

Chlorophyll Kinetics of Different Lines









39 winter and 33 summer lines were investigated.

Winter lines contain generally lower chlorophyll.

Here are kinetics of 4 summer lines, which are quite different.

There is a potential for further classical breeding activities !

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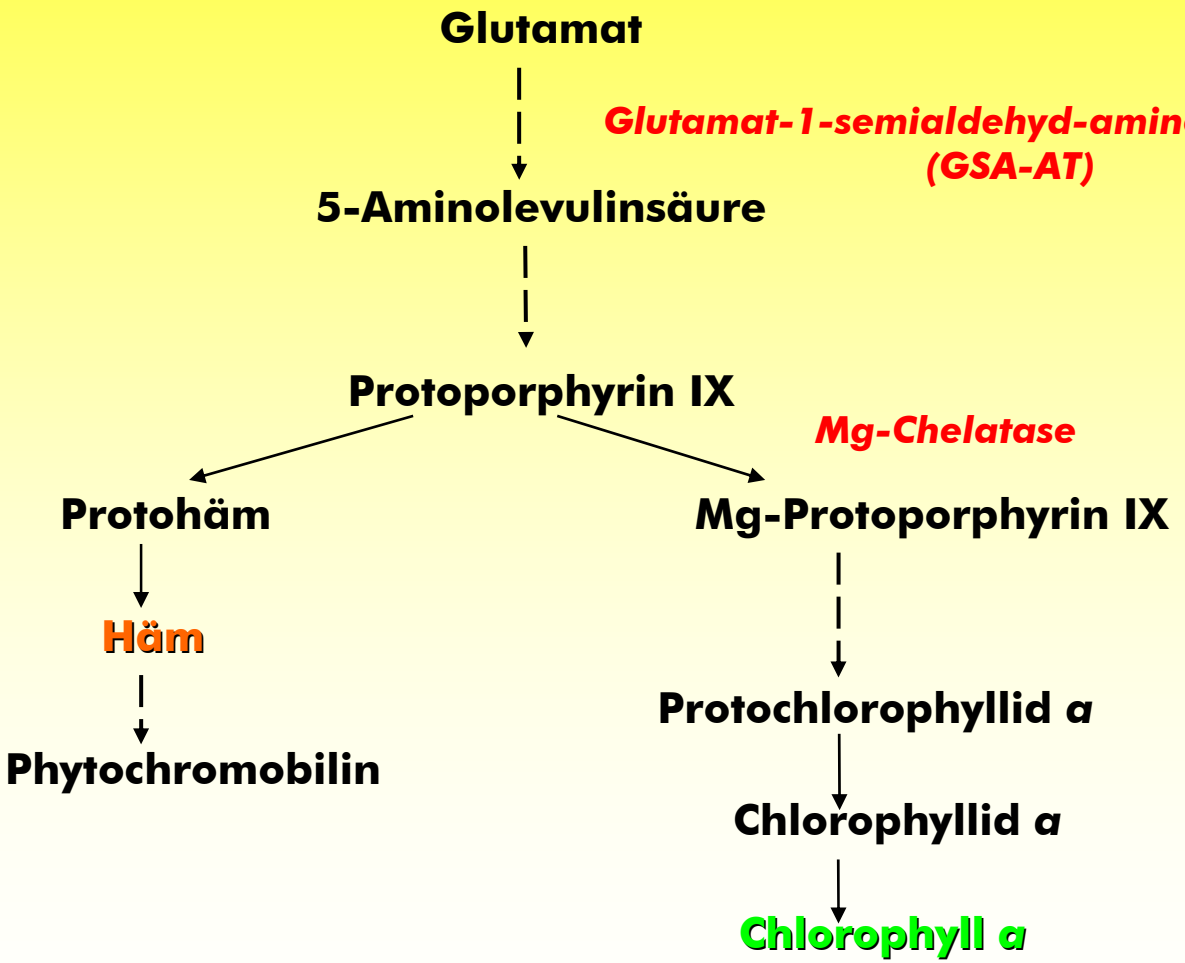
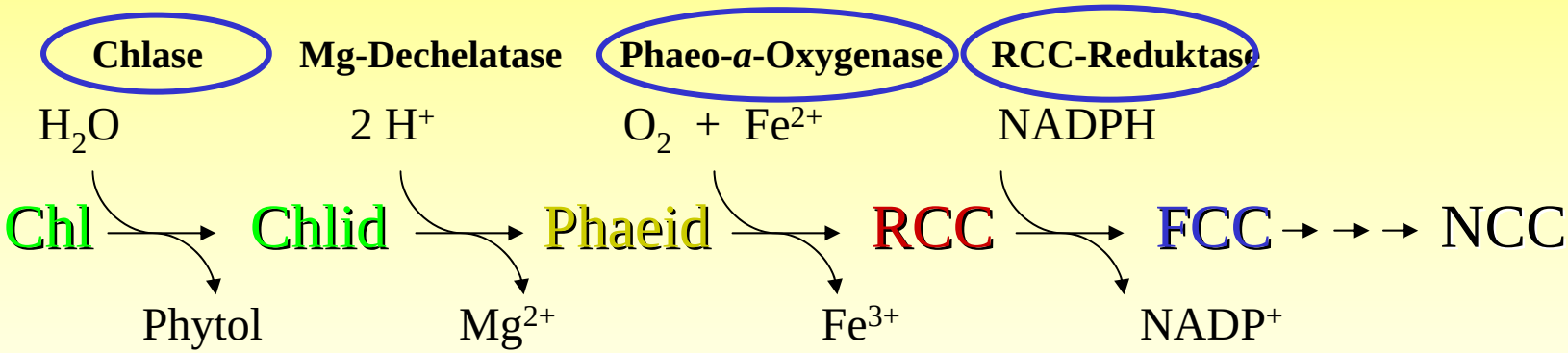
Chlorophyll Biosynthesis



Chlorophyll Catabolism



Genetic Engineering Scheme

- **Inactivation of chlorophyll producing enzymes**
  - **GSA-AT, Mg-chelatase**
    - **gene amplification / PCR-isolation**
    - **gene constructs by RNA interference**
    - **rape hypocotyl transformation by *agrobacterium tumefaciens***
- **Overexpression of chlorophyll degrading enzymes**
  - **Chlorophyllase (from *citrus clementii*)**
  - **Phaeophorbide-a-oxygenase (from *tomato*)**
  - **RCC-reductase (delivered by University of Bern)**

Examples of 39 Transgenic Plants






Gene Construct	Expected Effect	Results
1-2 (GSA-AT-RNAi)	Inhibition of chlorophyll synthesis	no
2-1 (Mg-Chelatase-RNAi)	Inhibition of chlorophyll synthesis	-
6-1 (Chlorophyllase)	Acceleration of chlorophyll metabolism	in 4 cases +
7-1 (Chlorophyllase without TP)	Acceleration of chlorophyll metabolism	

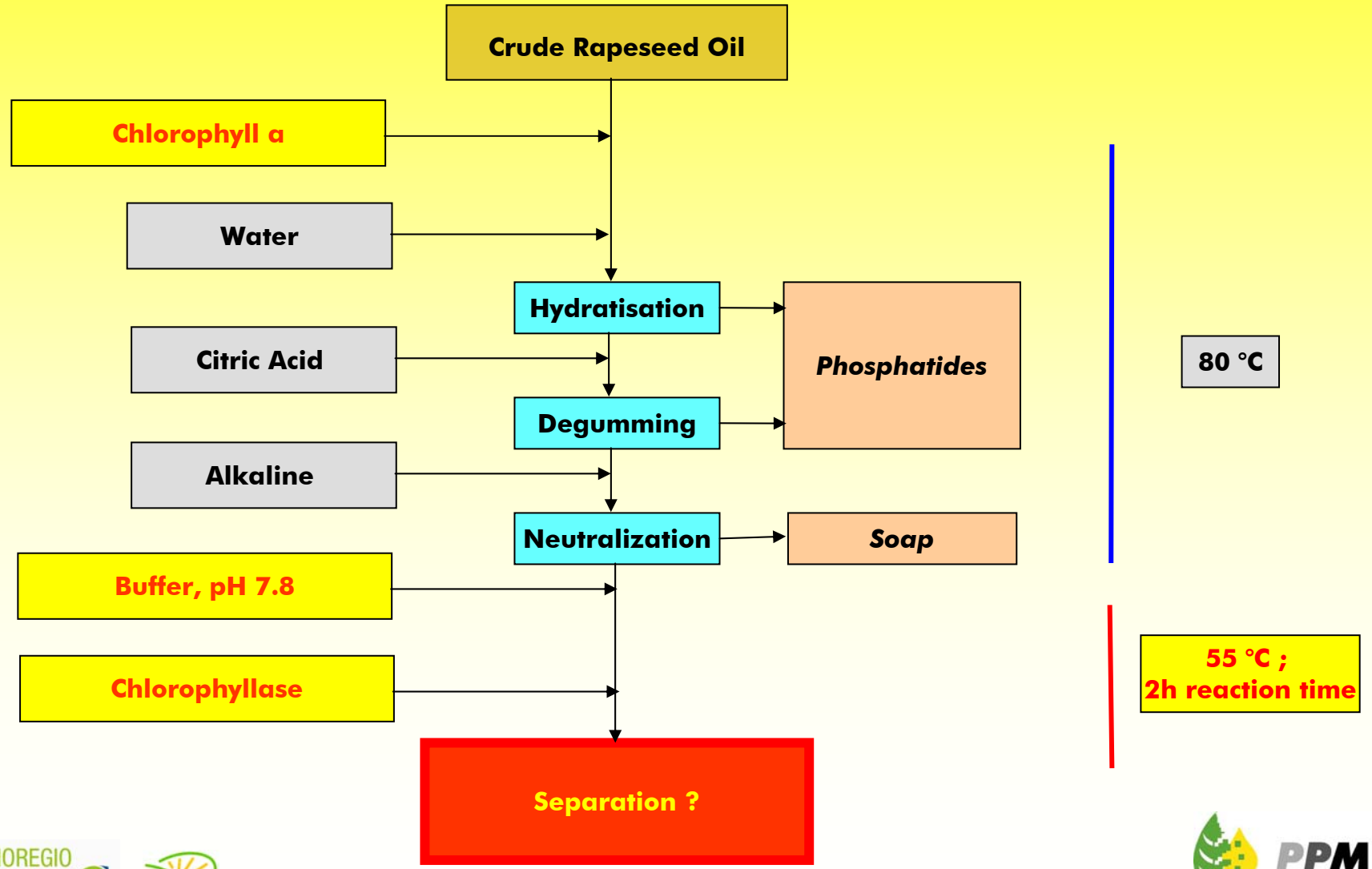
In 4 plants with chlorophyllase overexpression gene constructs reduced chlorophyll contents at the 42 day after pollination were measured.

Recombinant Proteins for Enzymatic Chlorophyll Removal from Oil

- **Chlorophyllase**
- **Phaeophorbid-*a*-oxygenase**
- **RCC-reductase**

- **Questions:**
  - **Are the enzymes active in oil?**
  - **Which are the optimal reaction conditions?**
  - **Where the enzymes are to put into the oil?**
  - **Are the enzymes able to harm the oil?**

Enzymatic Refining Test



Results of Enzymatic Refining Test

- **Recombinant chlorophyllase from *Citrus cl*. is able to split the chlorophyll into chlorophyllide and phytol under normal edible oil refining conditions.**

- **But, for the separation of the hydrophilic chlorophyllide an acetonic system is necessary.**

- **The activity of recombinant pheaophorbide-a-oxygenase and RCC-reductase is proved in vitro. Because of the need of some co-factors, their application in an edible oil refining process is not to expect.**

Conclusions

- **Even low reductions of the chlorophyll content in crude rapeseed oil lead to a considerable decrease in refining expenses, especially to a lower bleaching earth consumption.**
- **Different chlorophyll reduction strategies were investigated.**
- **The chlorophyll kinetics of 33 summer and 39 winter rapeseed lines during ripening were measured. Large differences between them indicate a potential for classical plant breeding of rapeseed lines with low chlorophyll content.**
- **There were 39 transgenic plants generated. 4 of them showed a significantly lower chlorophyll content at the 42. day after pollination.**
- **An enzymatic refining process by the use of recombinant proteins for chlorophyll removing is not to expect.**

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Thank You

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