



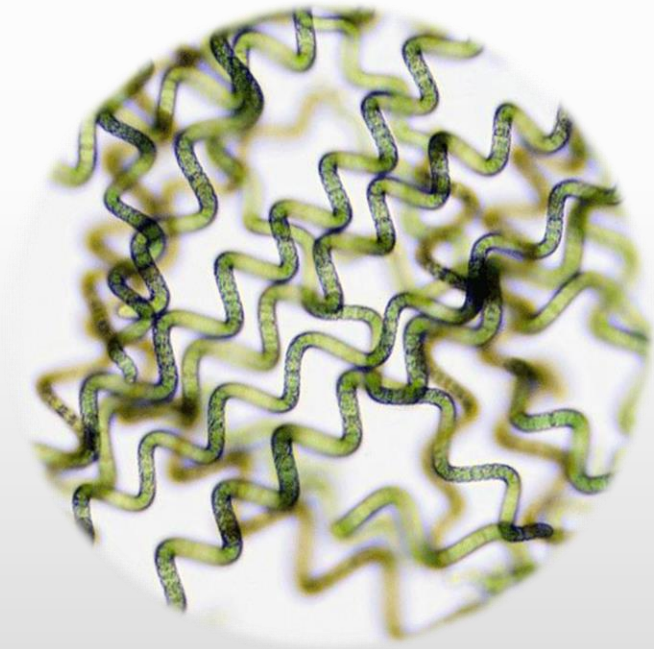
# Fermented *Spirulina* Extract Antioxidant Activity in vitro and at the Cellular and Proteome Level

*J. Masten Rutar, B. Cillero-Pastor, R.  
Mohren, L. Pogačnik, N. Poklar Ulrih,  
N. Ogrinc, P. Jamnik*



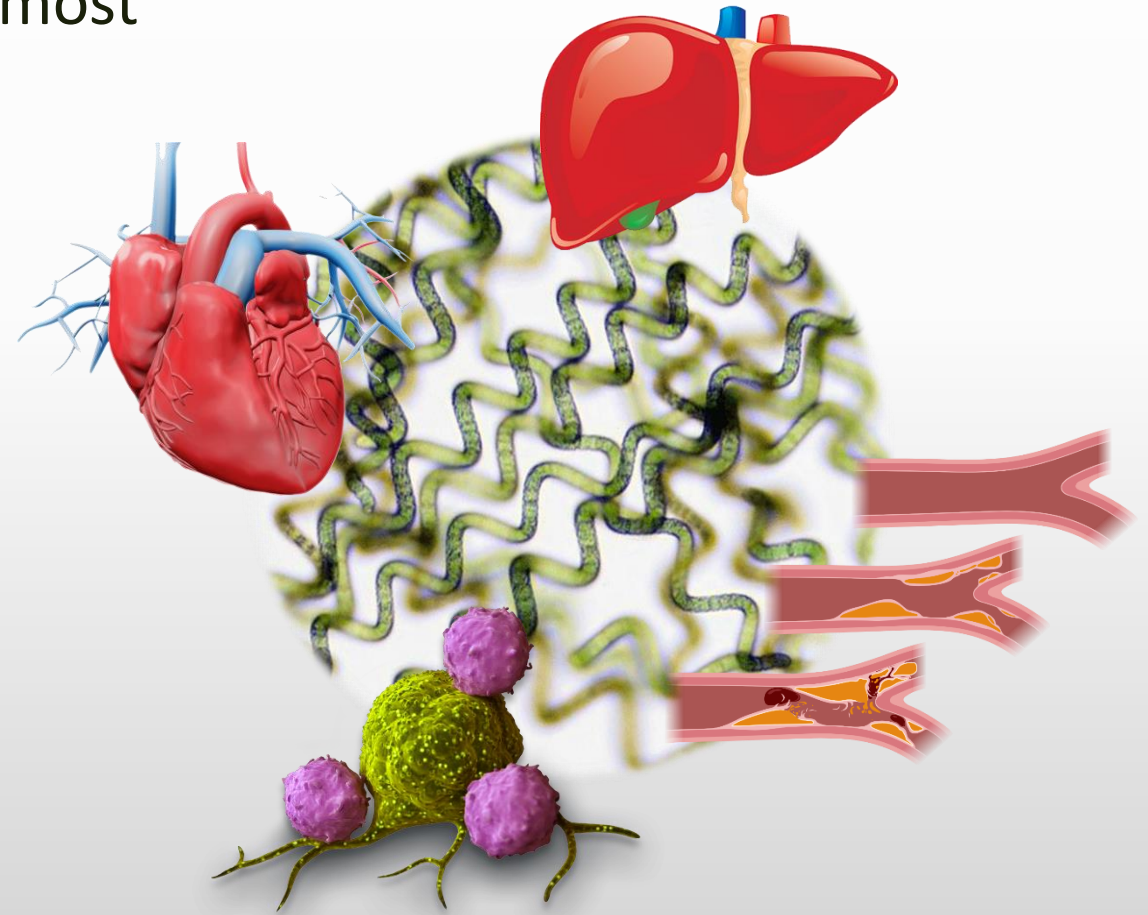
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- Contains macro- and micronutrients:



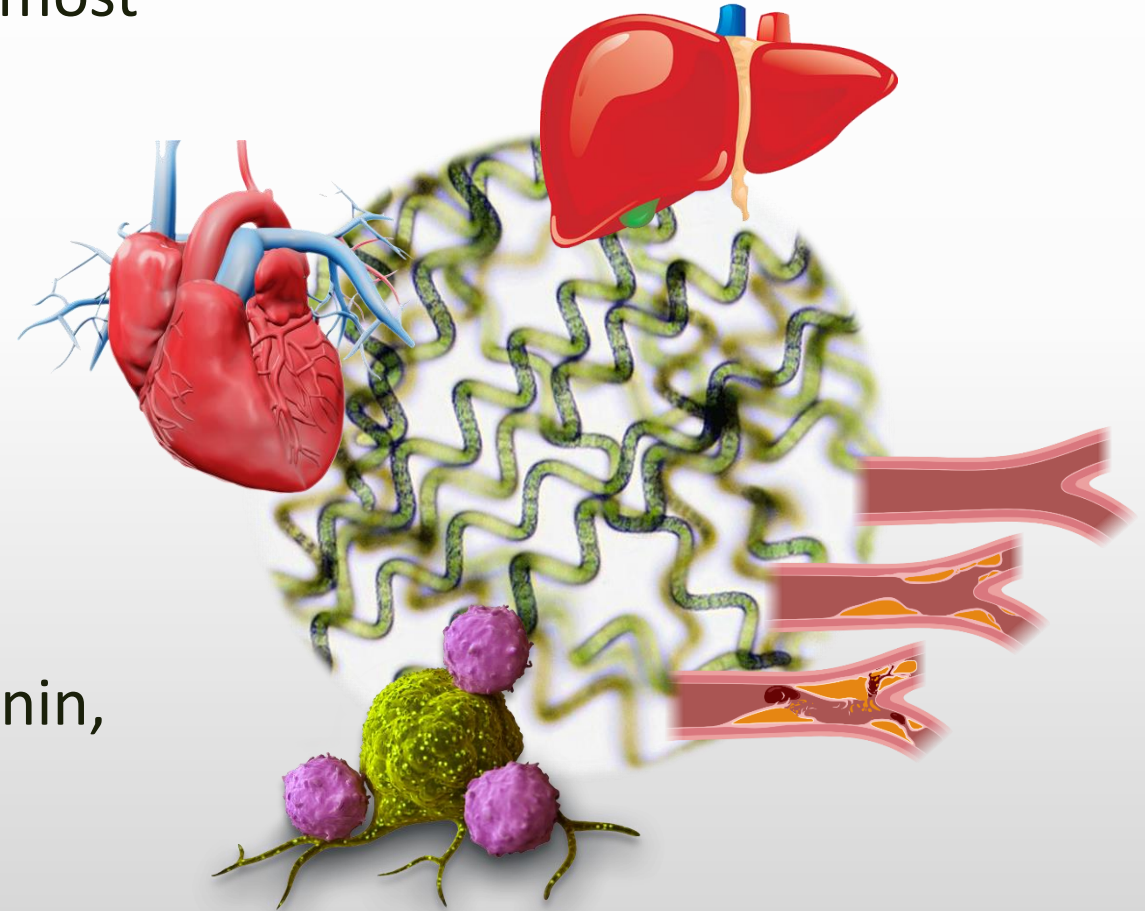
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- Contains macro- and micronutrients:
  - High-quality proteins
  - Minerals
  - Vitamins
  - Fatty acids
  - Polysaccharides
  - Pigments (carotenoids, c-phycoerythrin, chlorophyll-a)
  - Other bioactive compounds

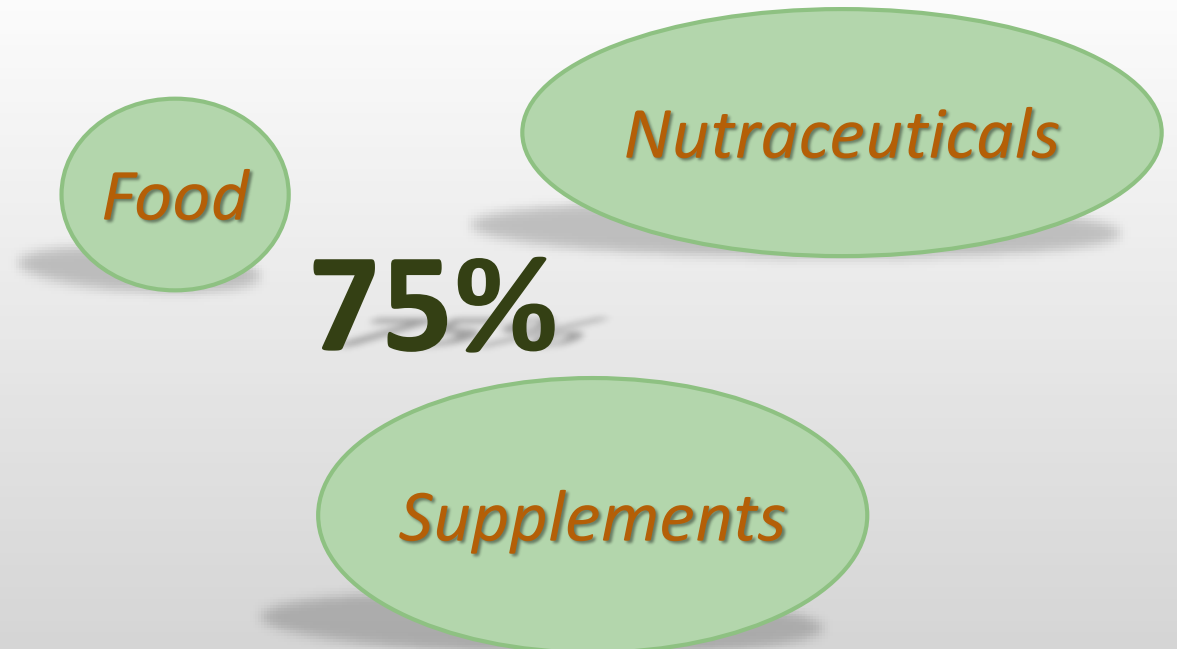


# *Spirulina* as nutritional supplement

- Spirulina product market: continued and rapid growth until 2028 (CAGR of 18.1%).
- Microalgae-based commercial products: Asia or Australia, European companies: 5% of the global market.

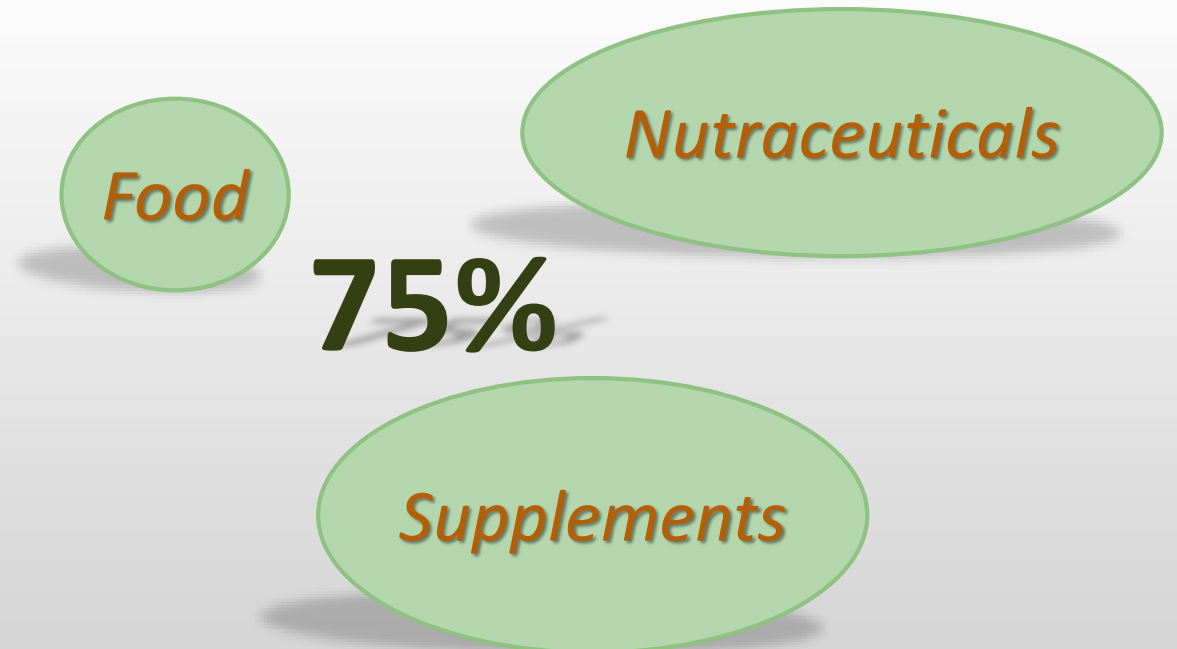
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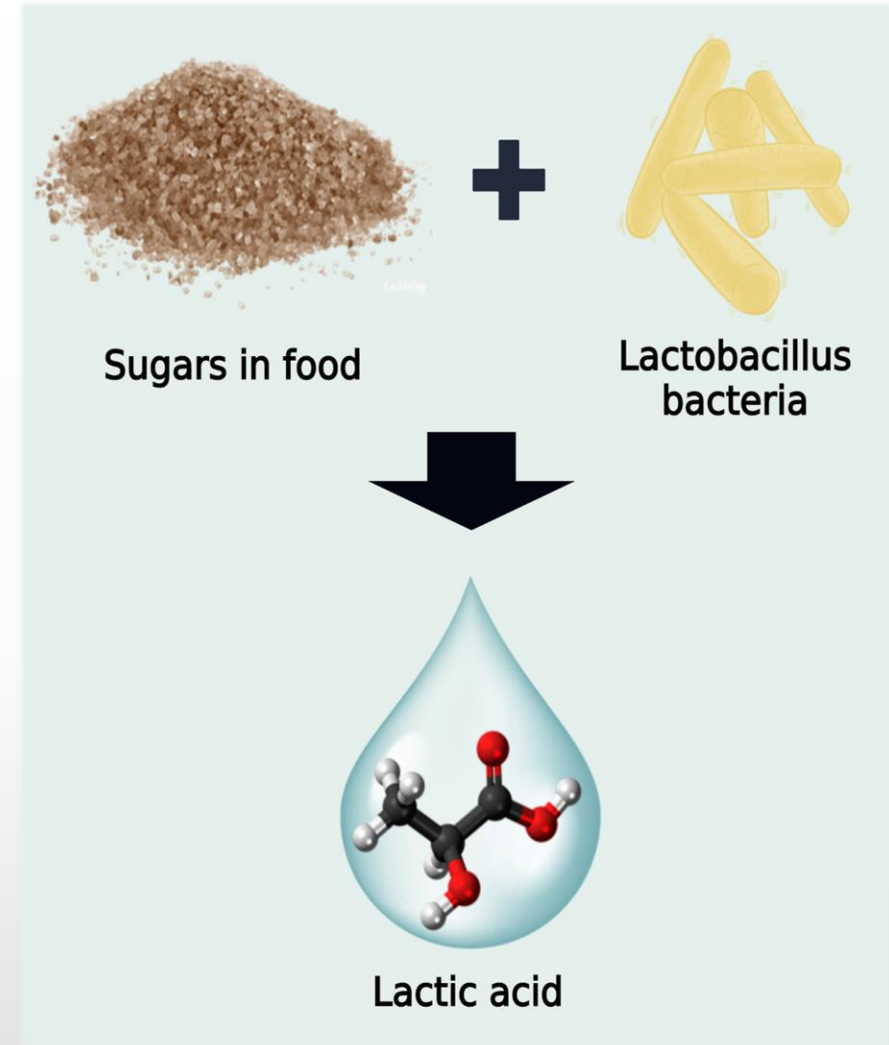
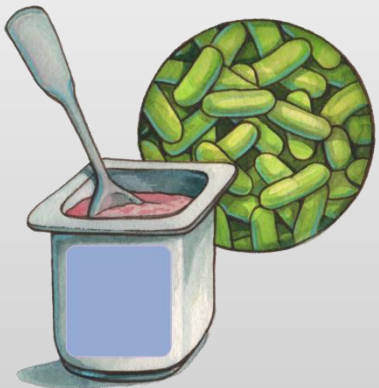
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- Microalgae-based commercial products: Asia or Australia, European companies: 5% of the global market.
- Increasing health awareness
- Vegetarianism
- Malnutrition
- Dietary supplement intake
- Demand for natural colorings



# Fermentation effect

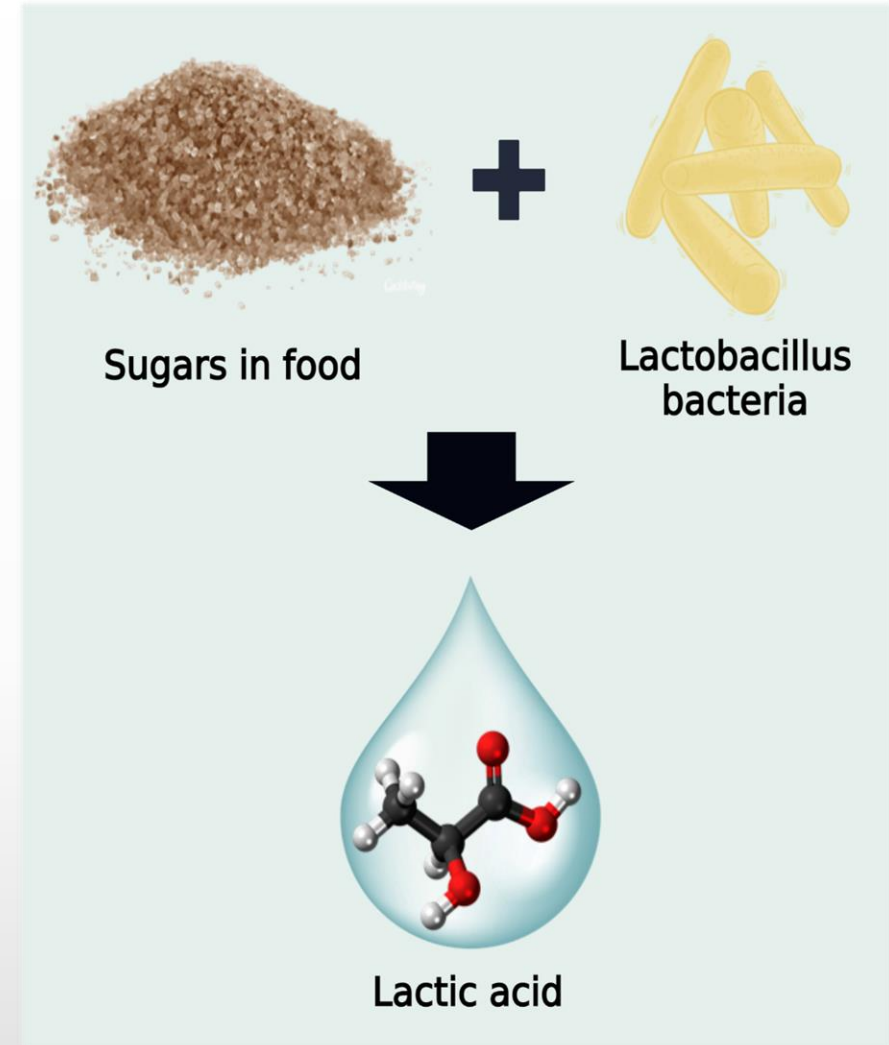
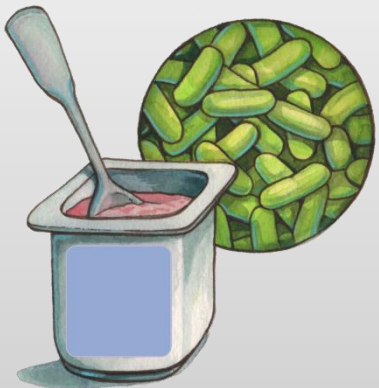
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- Degradation of cyanobacterial cell walls
- Improved bioaccessibility and bioavailability
- Molecules with high:





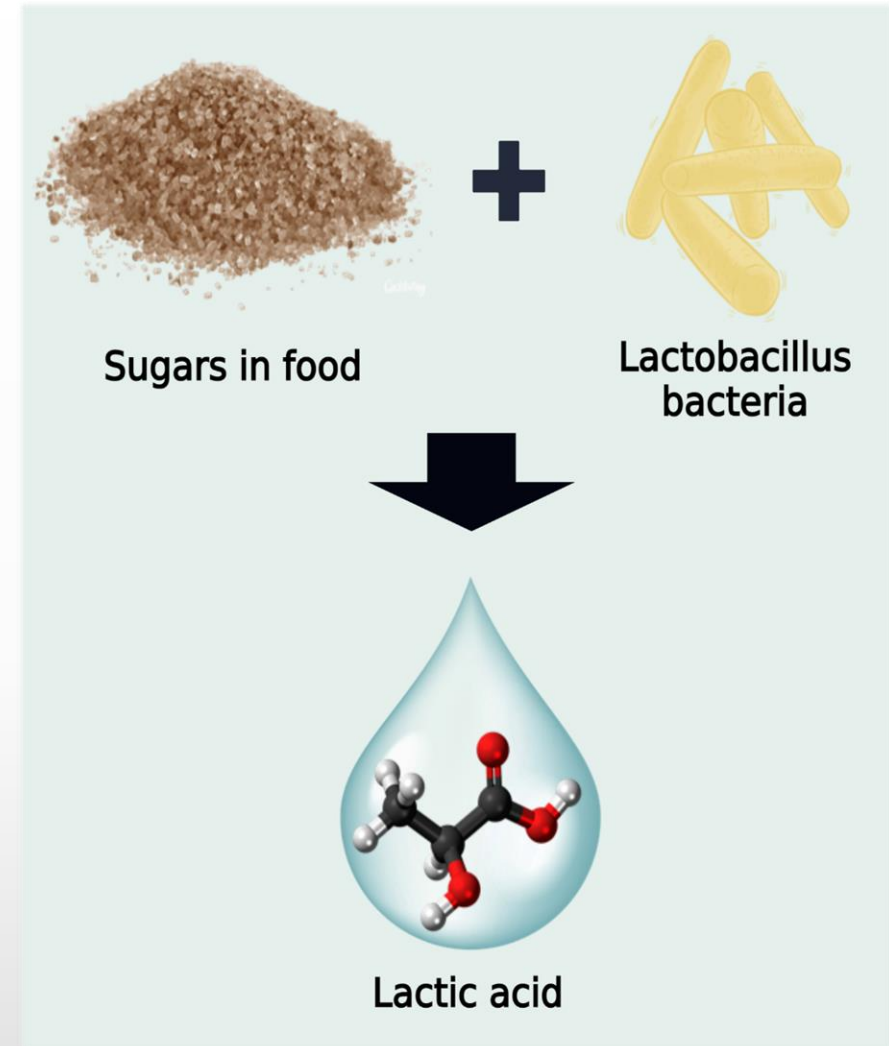
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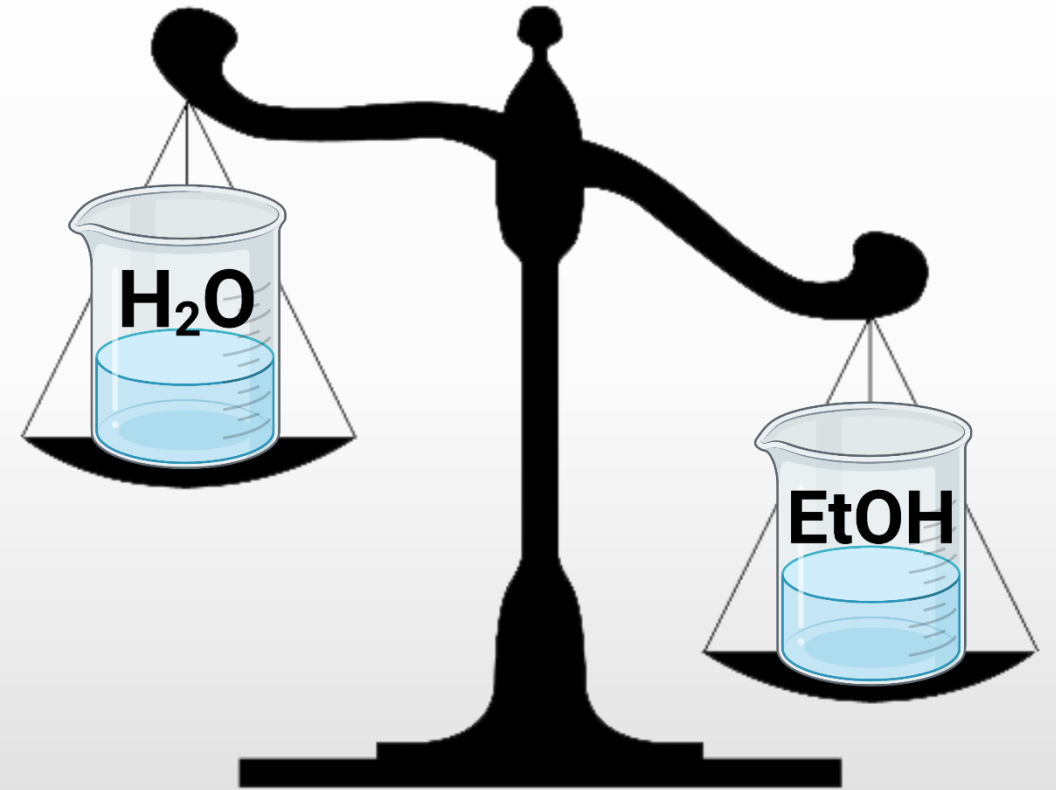
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Better nutritional and functional characteristics

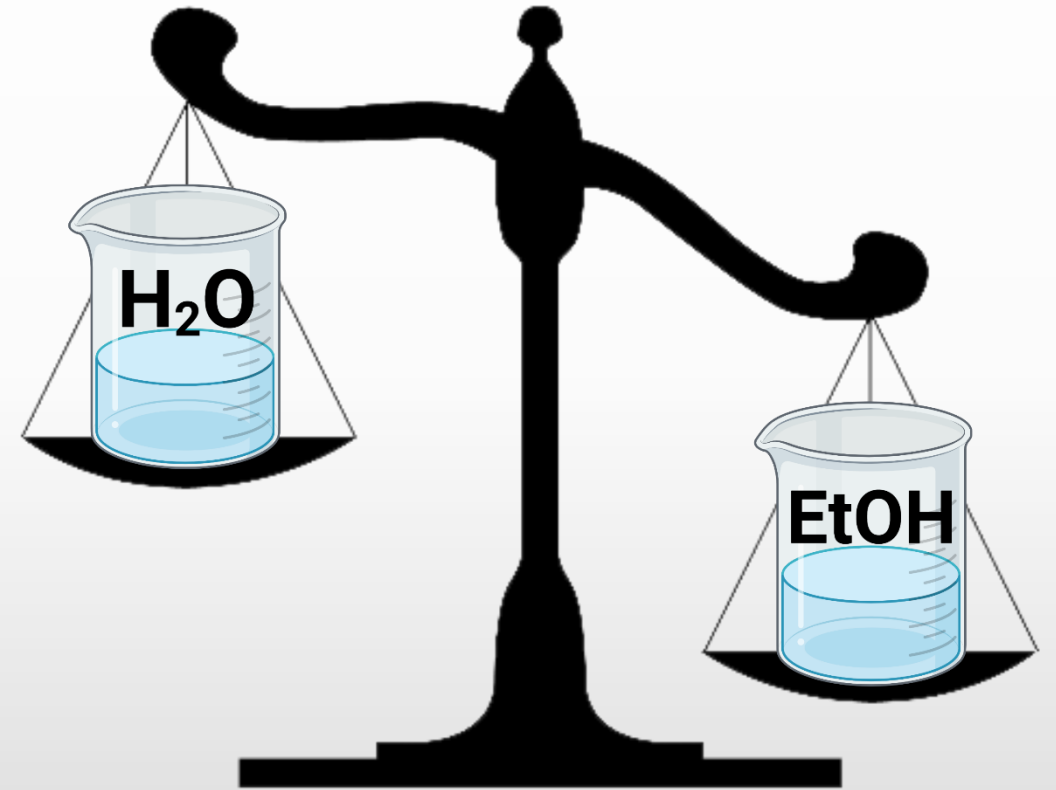
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- Alcohol-soluble components: main antioxidative properties



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- **Higher antiradical and antioxidant activity!**



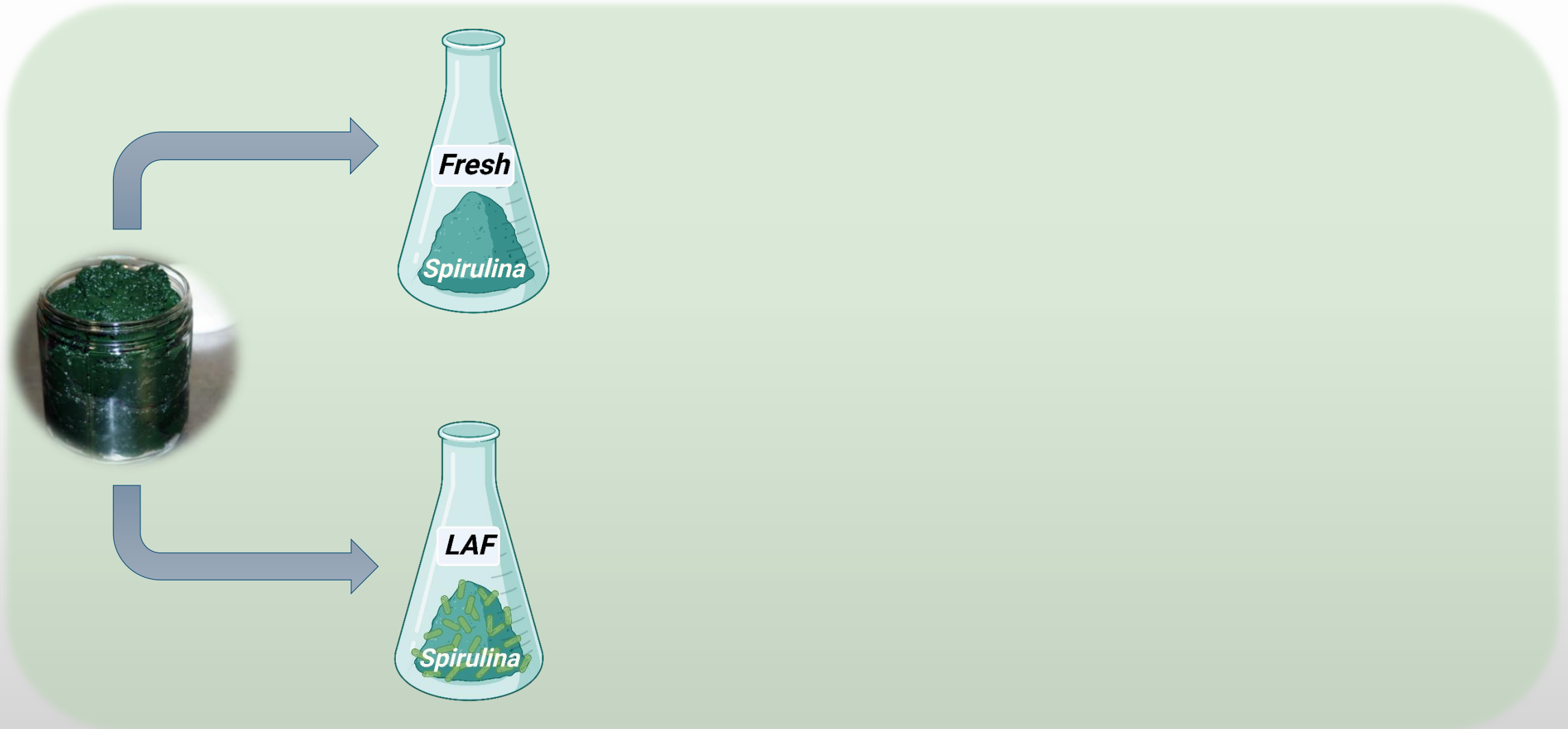
# Sample preparation and analysis



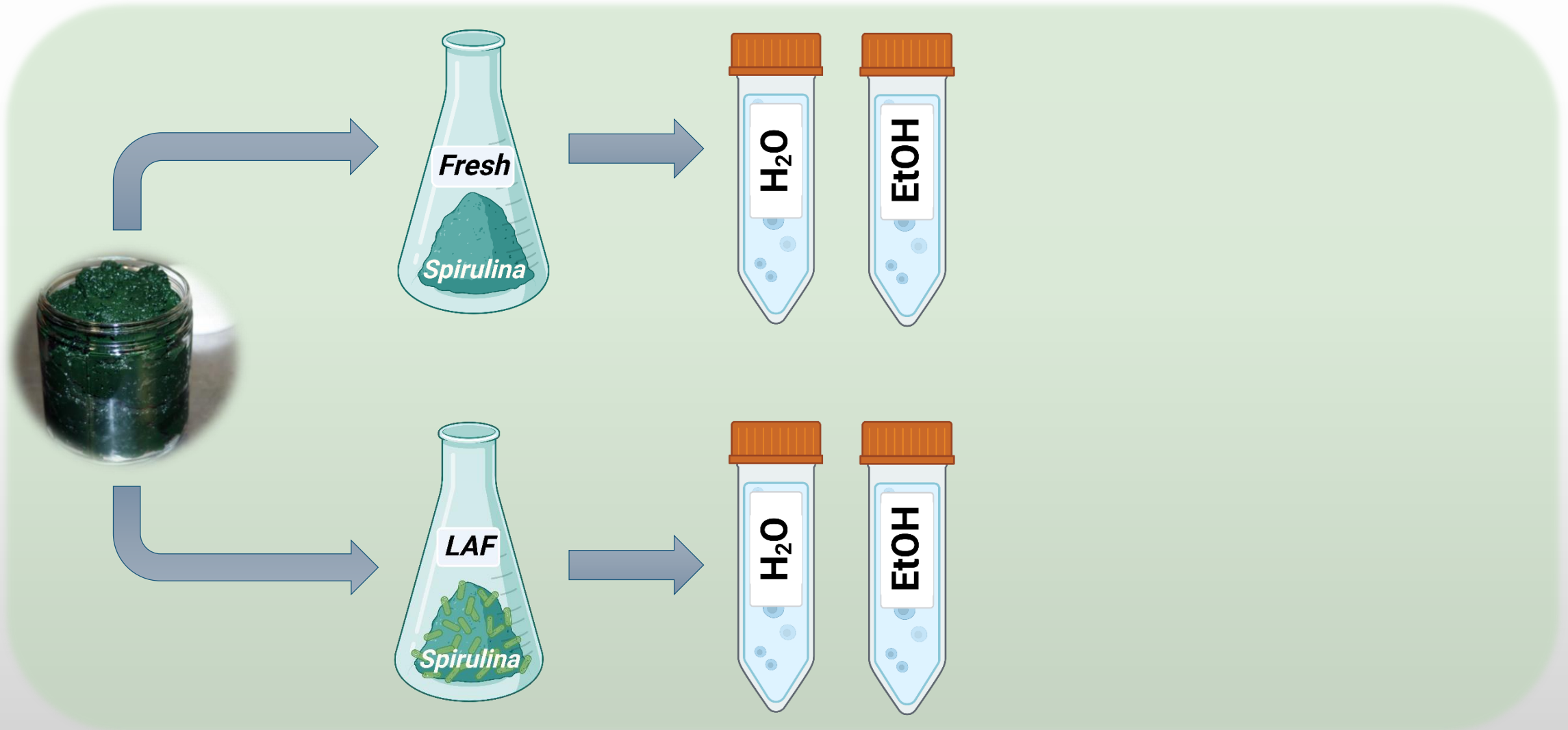
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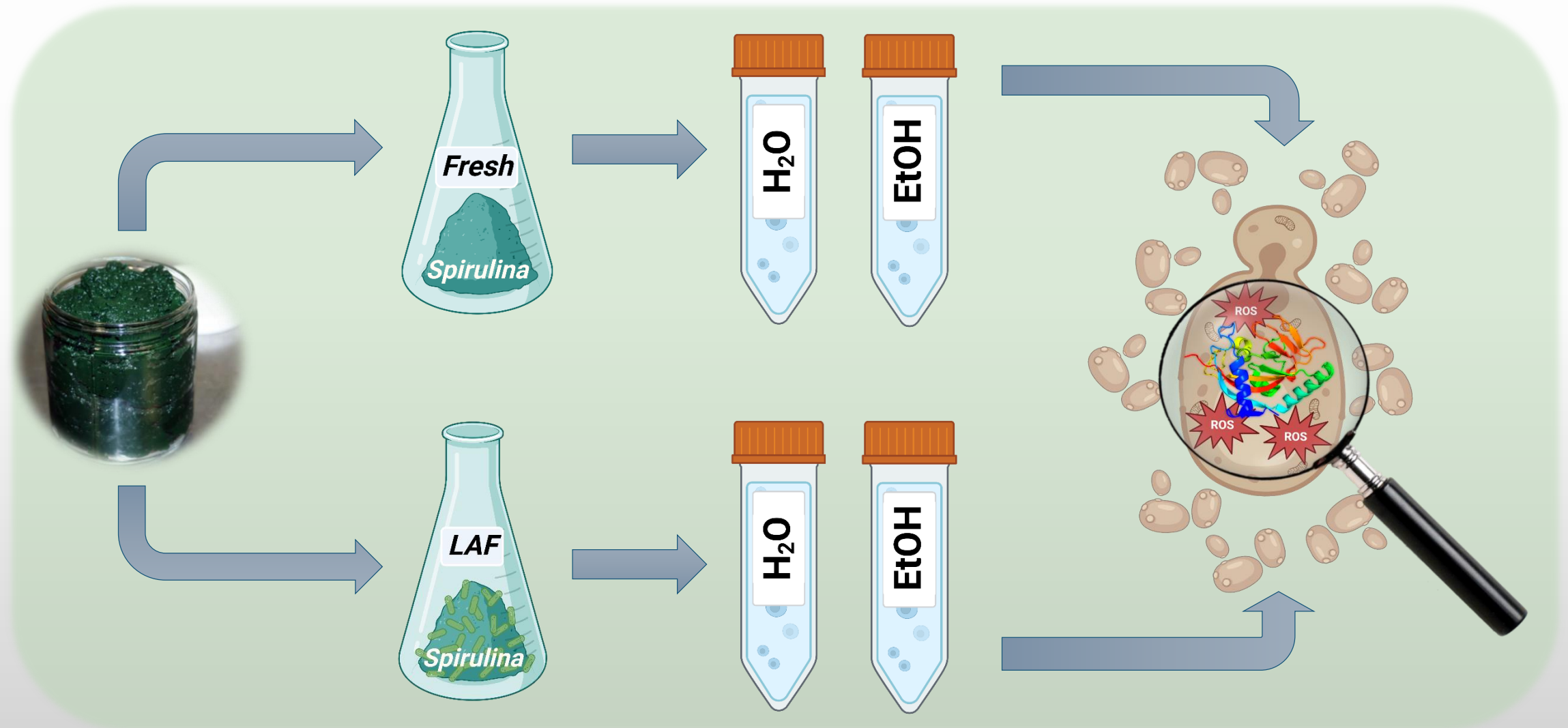


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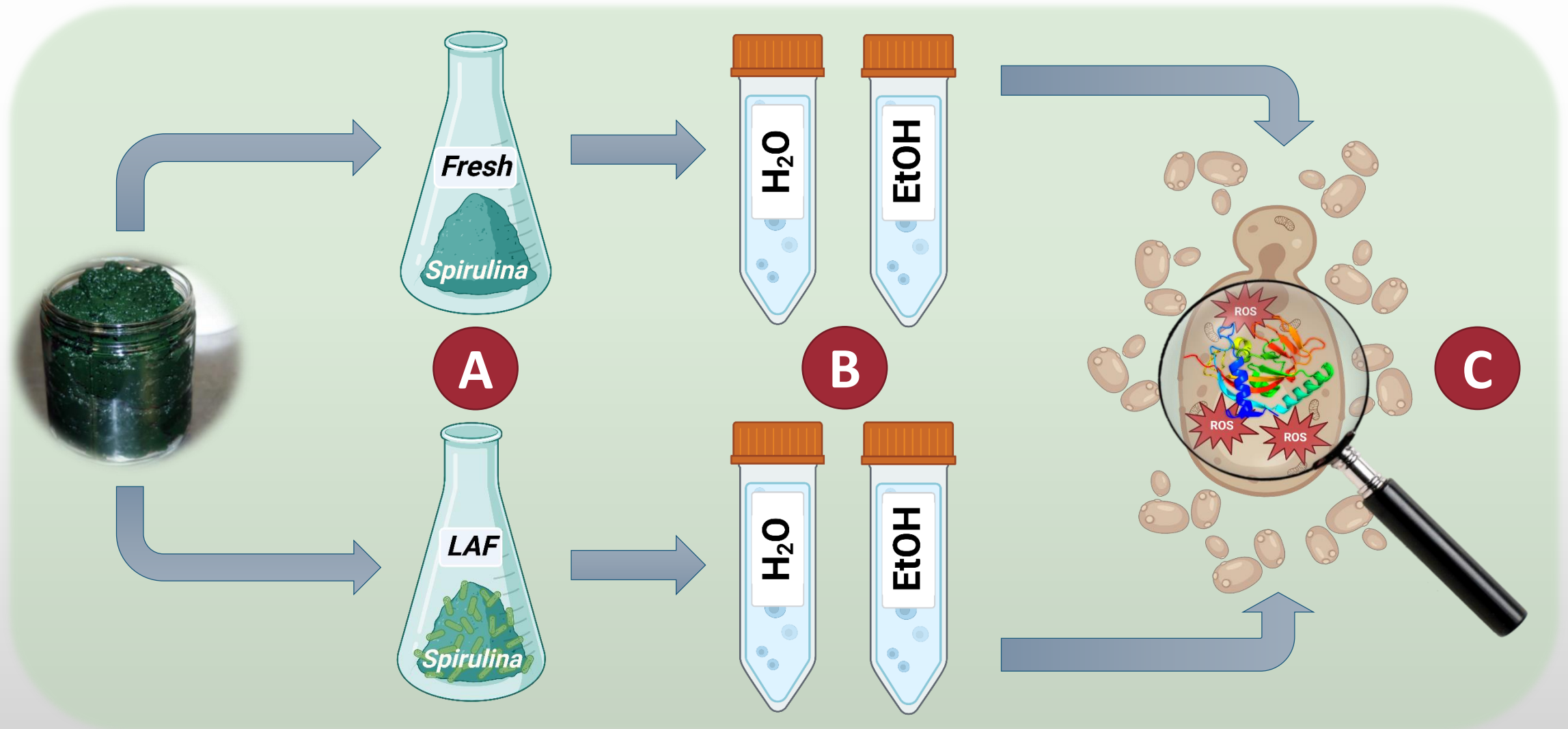




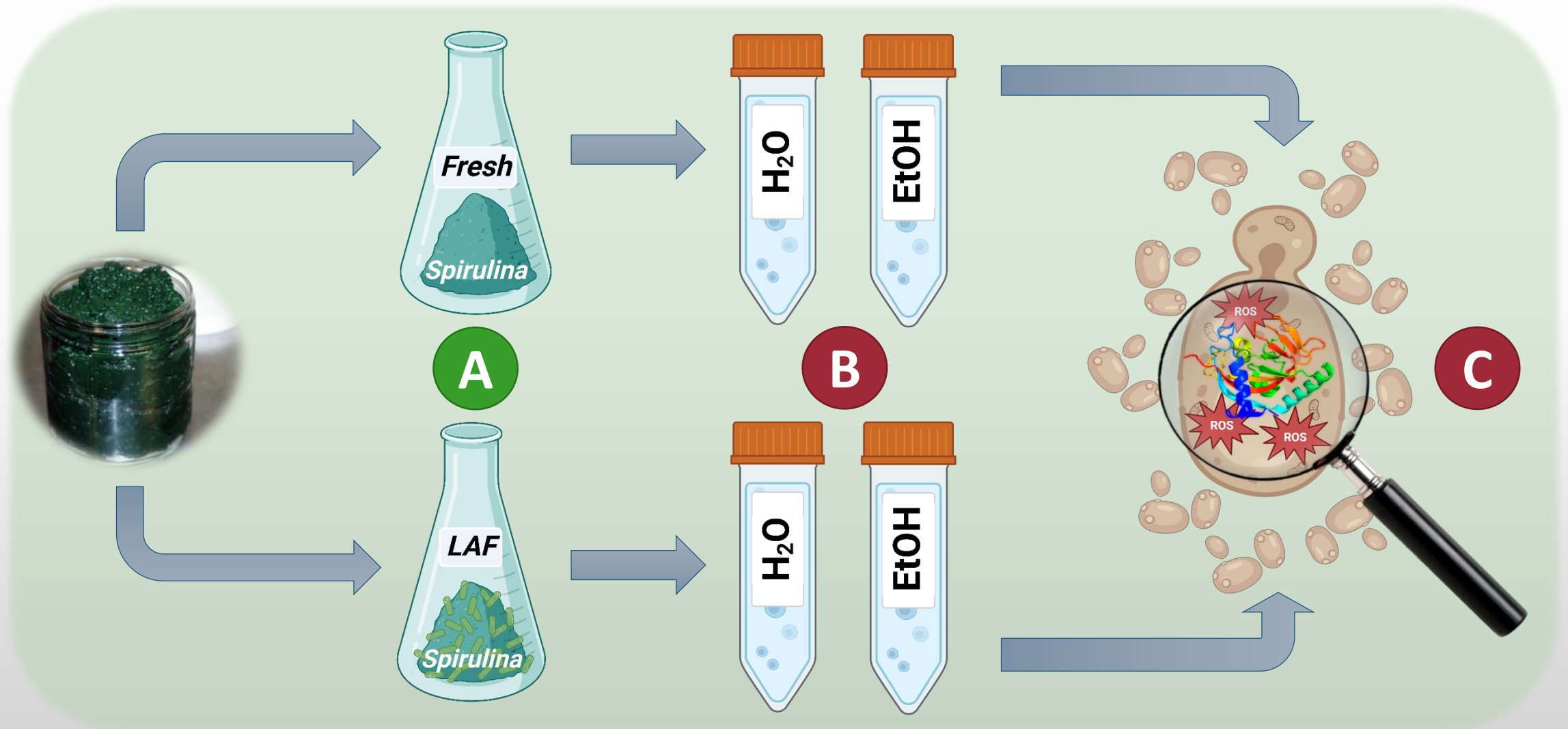
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# A: Fermented and non-fermented broth

Component	NFB	FB
Crude protein	46.56 ± 2.43	47.37 ± 1.49
Total ash	12.65 ± 0.34	12.78 ± 0.08
Crude fat	<b>6.26 ± 0.04</b>	<b>6.00 ± 0.01</b>
Soluble dietary fibres	3.20 ± 0.46	4.02 ± 0.69
Insoluble dietary fibres	19.33 ± 0.70	17.79 ± 0.94
Total dietary fibres	22.53 ± 0.38	21.81 ± 0.72
Available carbohydrates	13.00 ± 0.74	11.05 ± 1.94
Non protein nitrogen/total nitrogen	<b>24.8% ± 1.5%</b>	<b>28.4% ± 1.1%</b>

- Non-protein nitrogen increased:

Higher protein bioavailability

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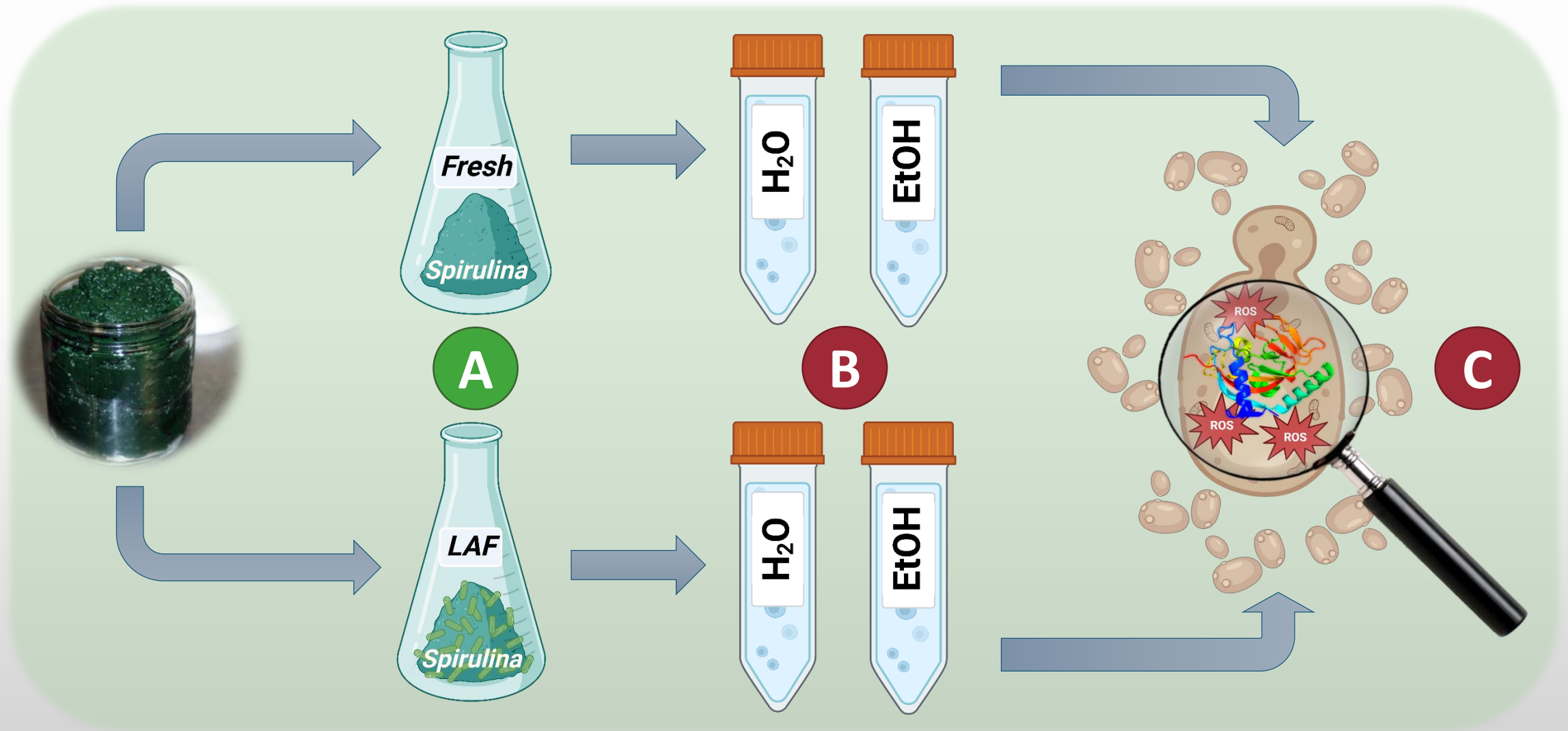
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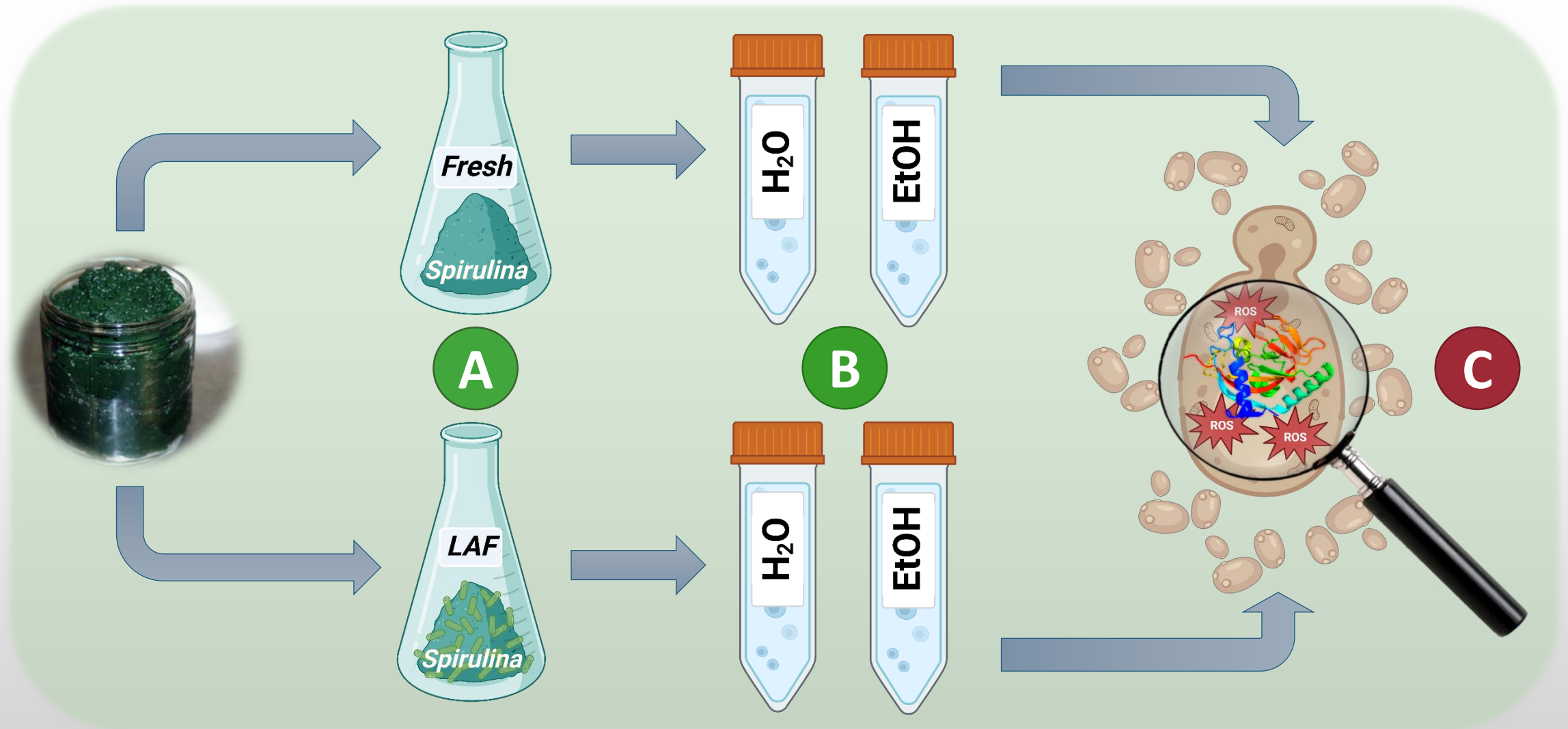
- Fat content decreased
- Other nutrients remained the same
- No pathogenic bacteria

Microorganisms	N (CFU/g) in NFB	N (CFU/g) in FB
Coliform bacteria	<10	<10
<i>Escherichia coli</i>	<100	<100
<i>Staphylococcus aureus</i>	<100	<100
<i>Bacillus cereus</i>	<100	<100
<i>Clostridium perfringens</i>	<100	<100
Yeast	<10	<10
Moulds	<10	<10
<i>Salmonella</i> spp. *	Neg. in 10 g	Neg. in 10 g
<i>L. monocytogenes</i> *	Neg. in 10 g	Neg. in 10 g

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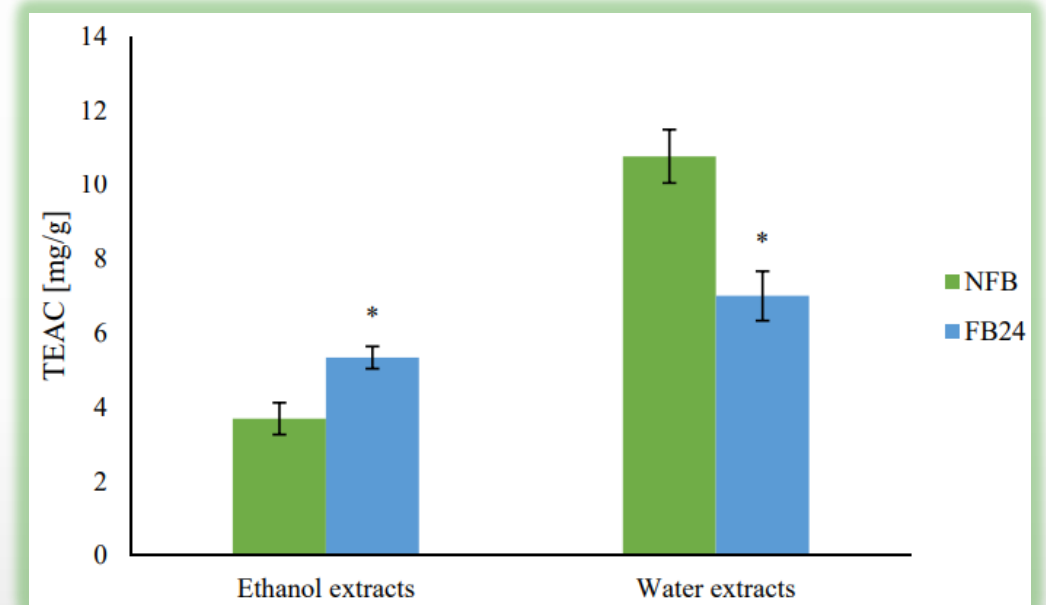
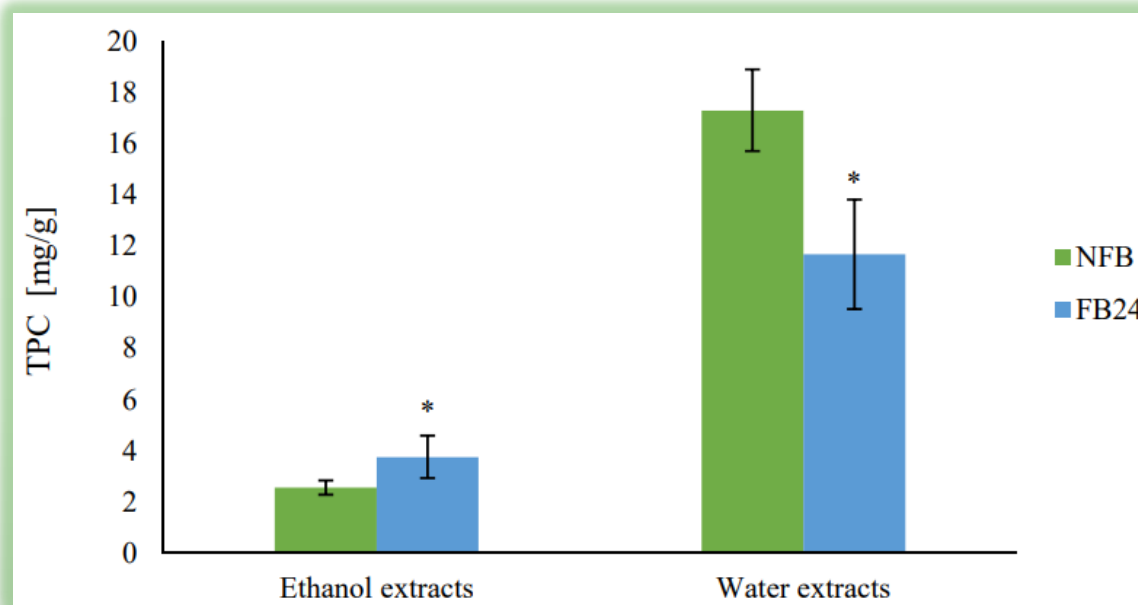


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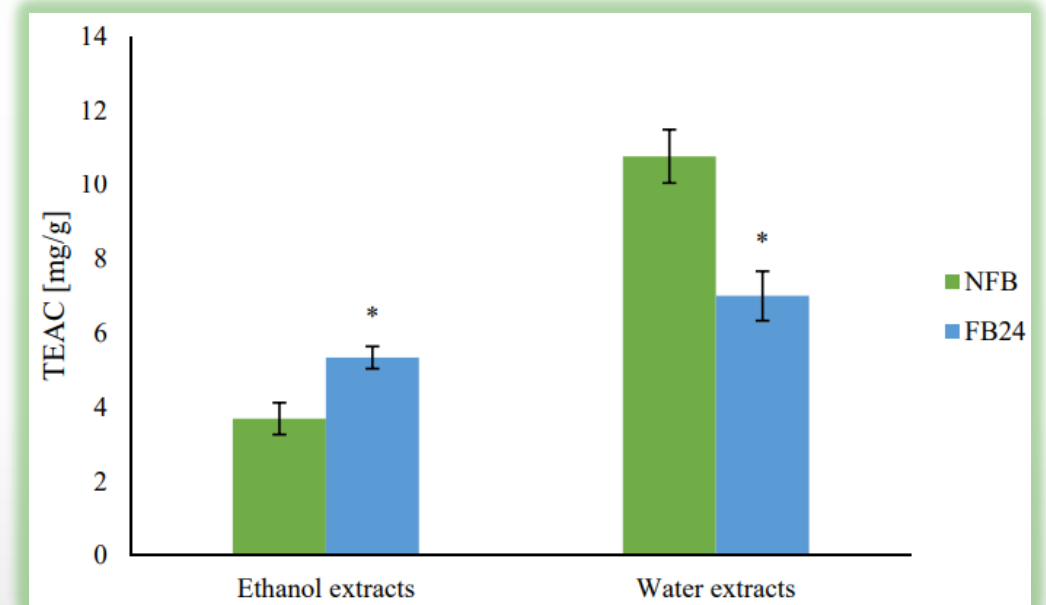
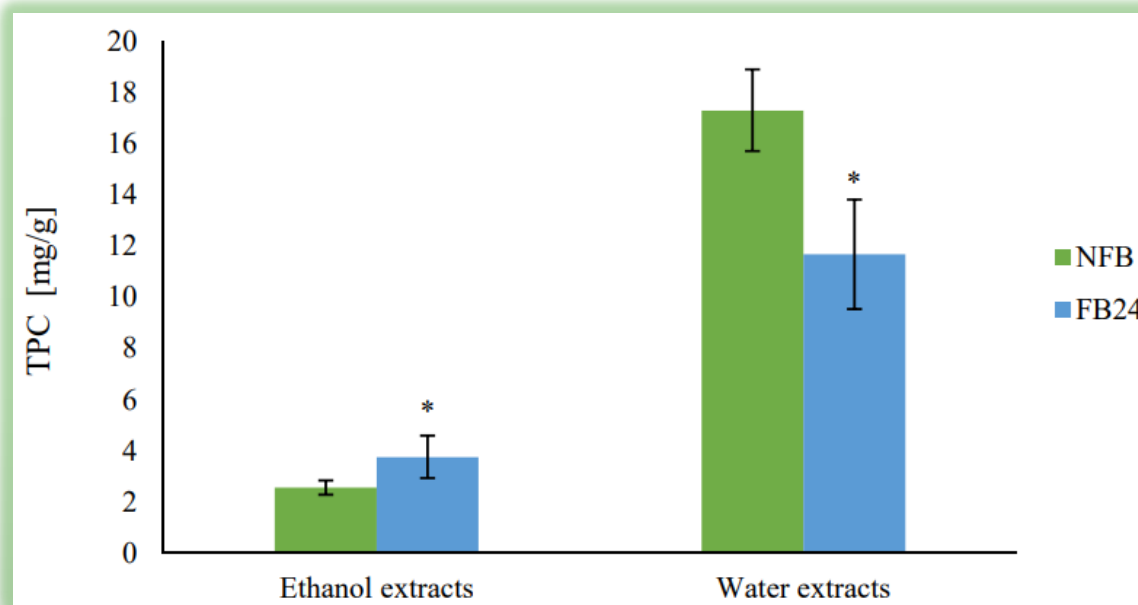


## B: Water and ethanol extracts



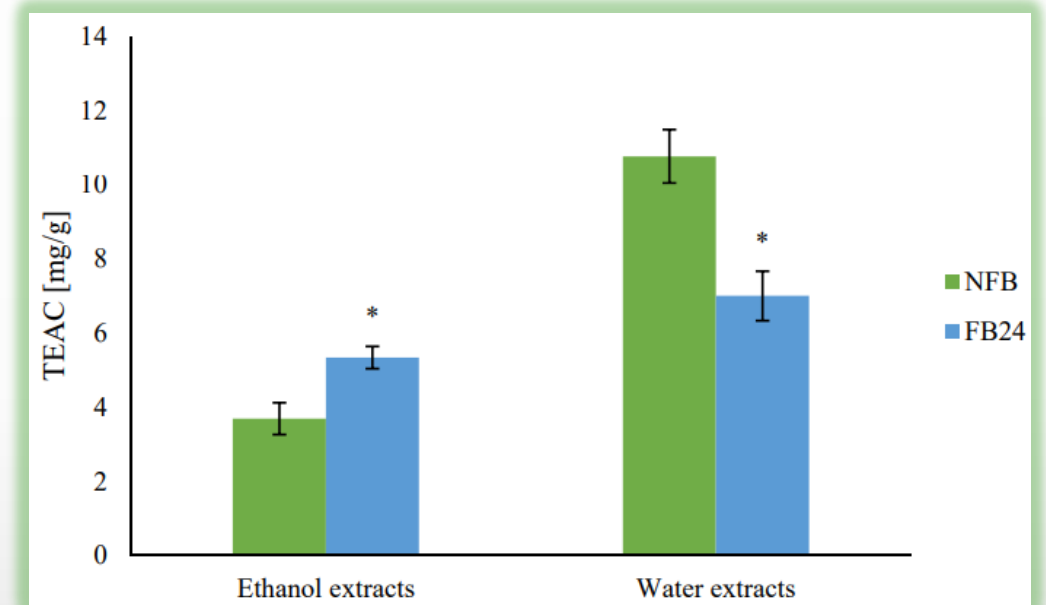
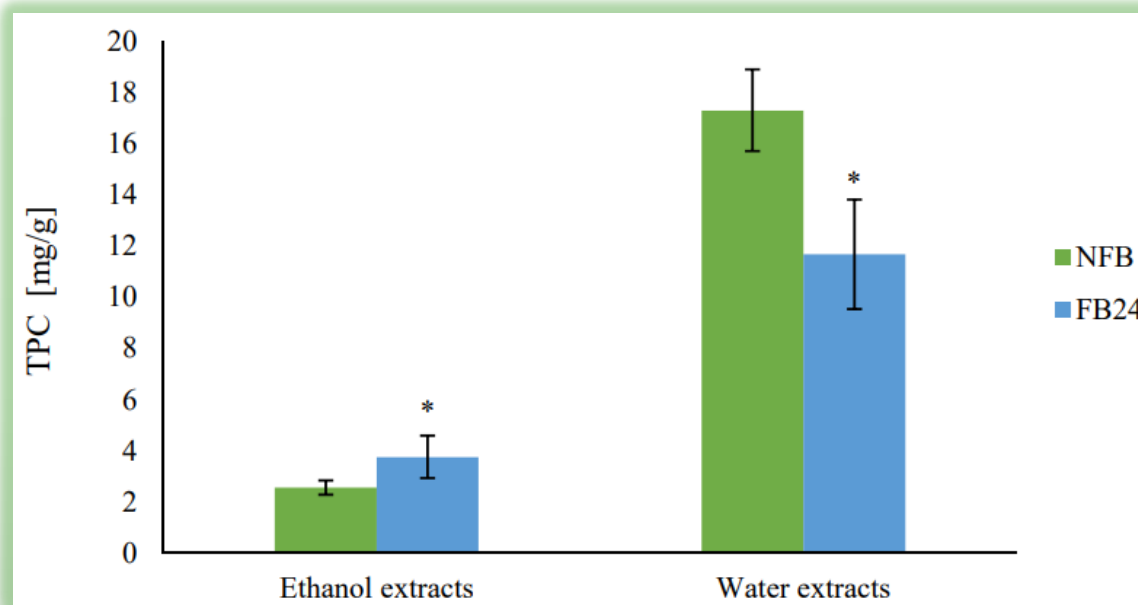
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- **Water extracts:** 33% decrease in TPC and 35% decrease in TEAC
- **Ethanol extracts:** 45% increase in TPC and 30% increase in TEAC

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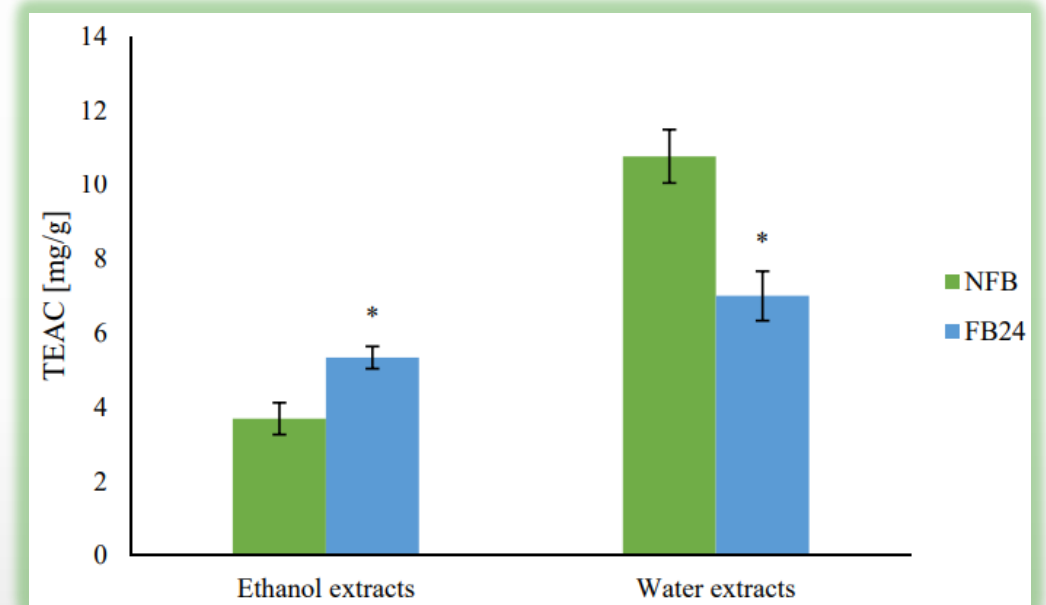
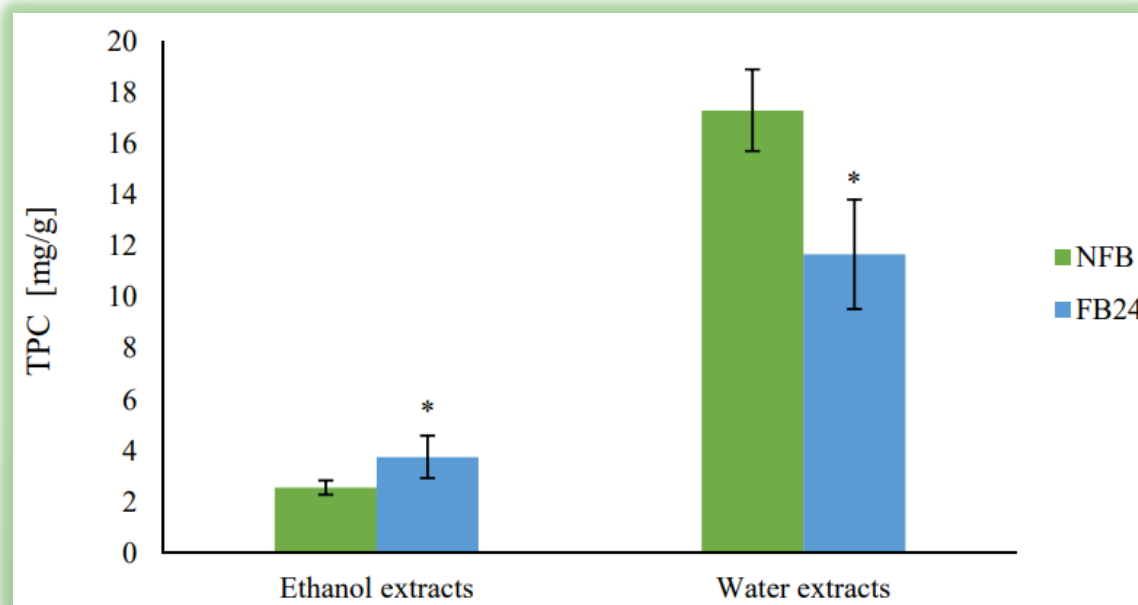
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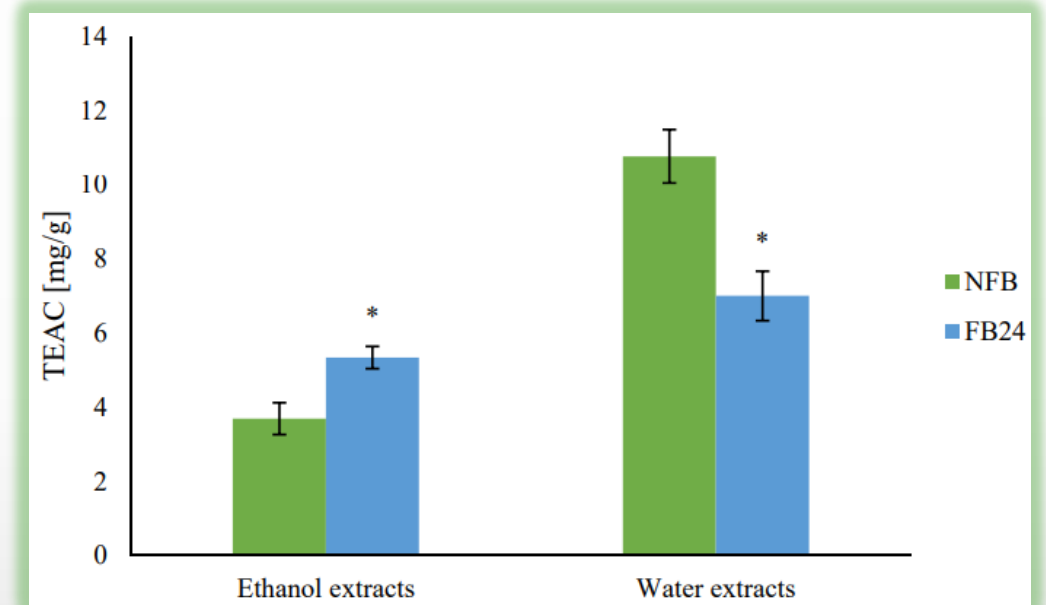
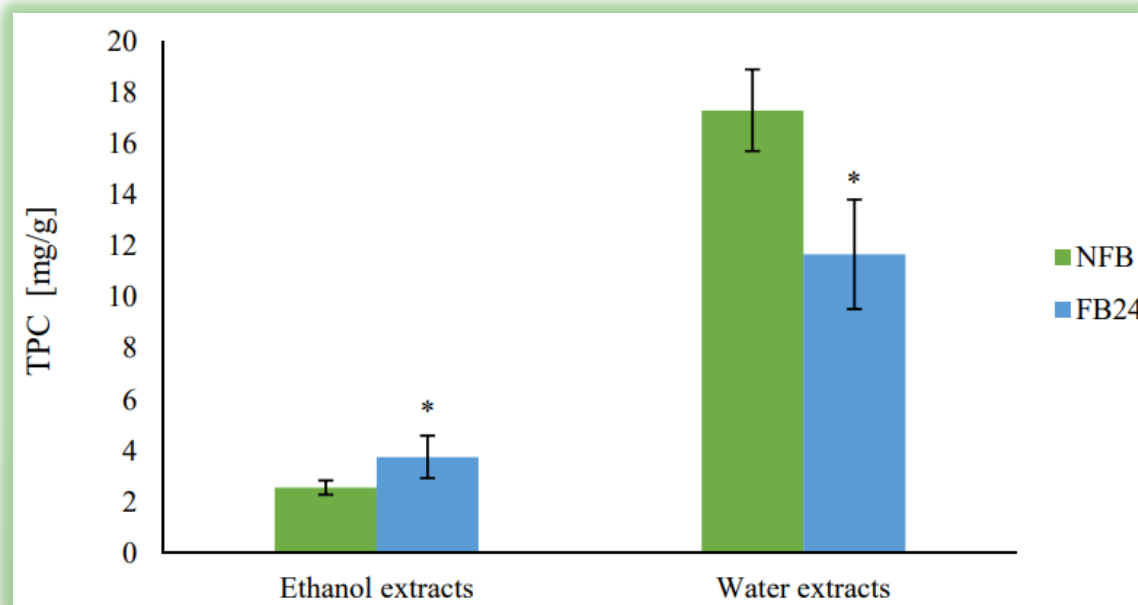
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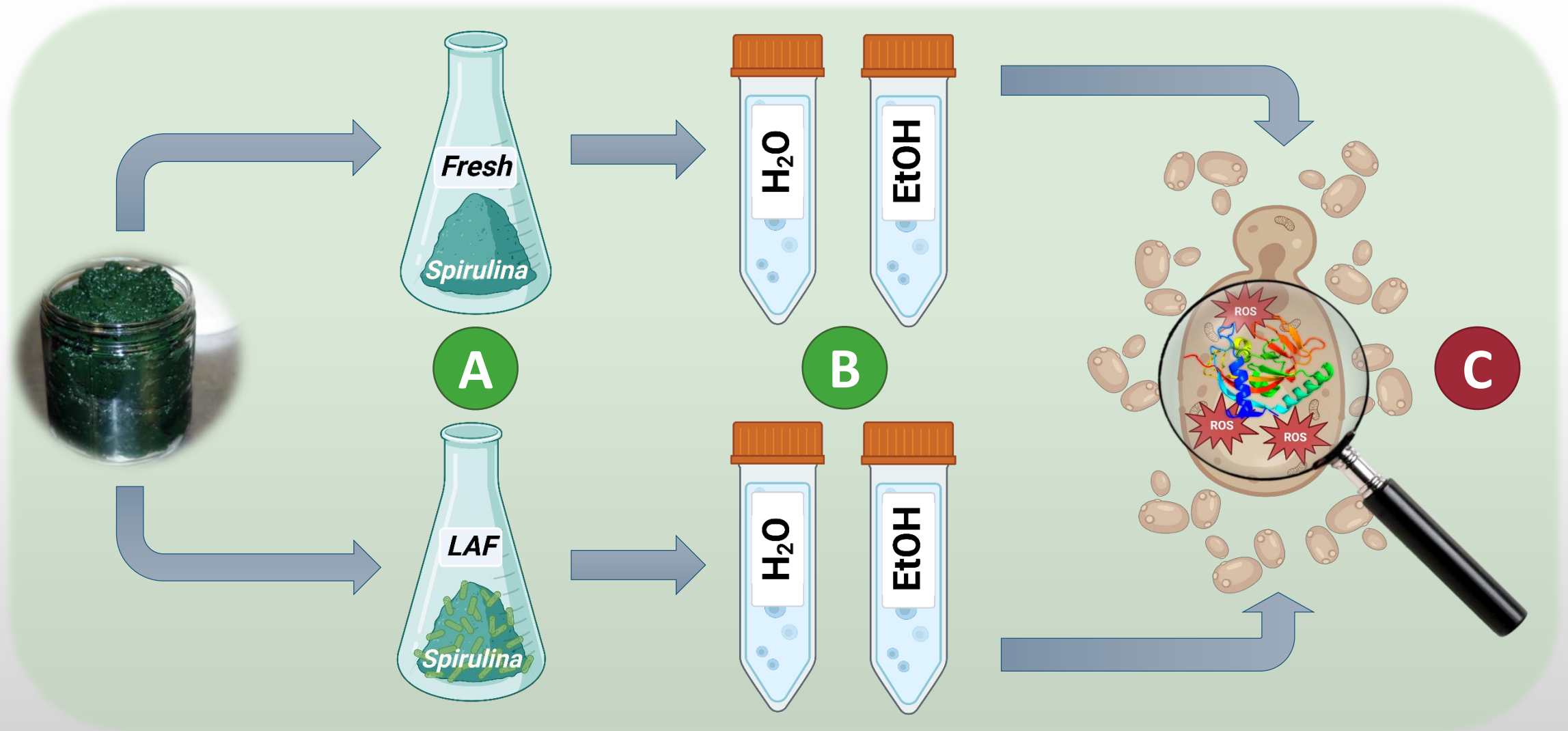
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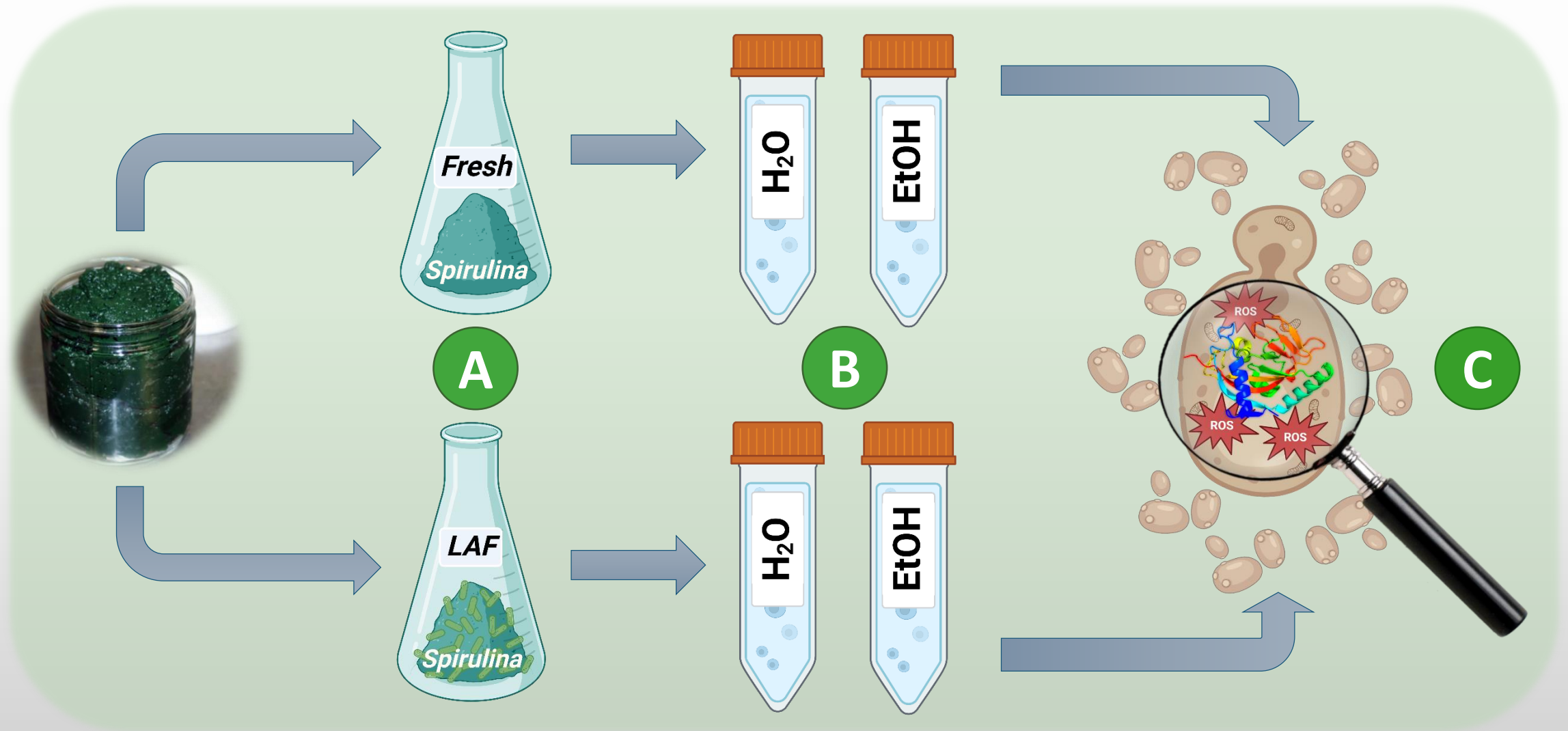


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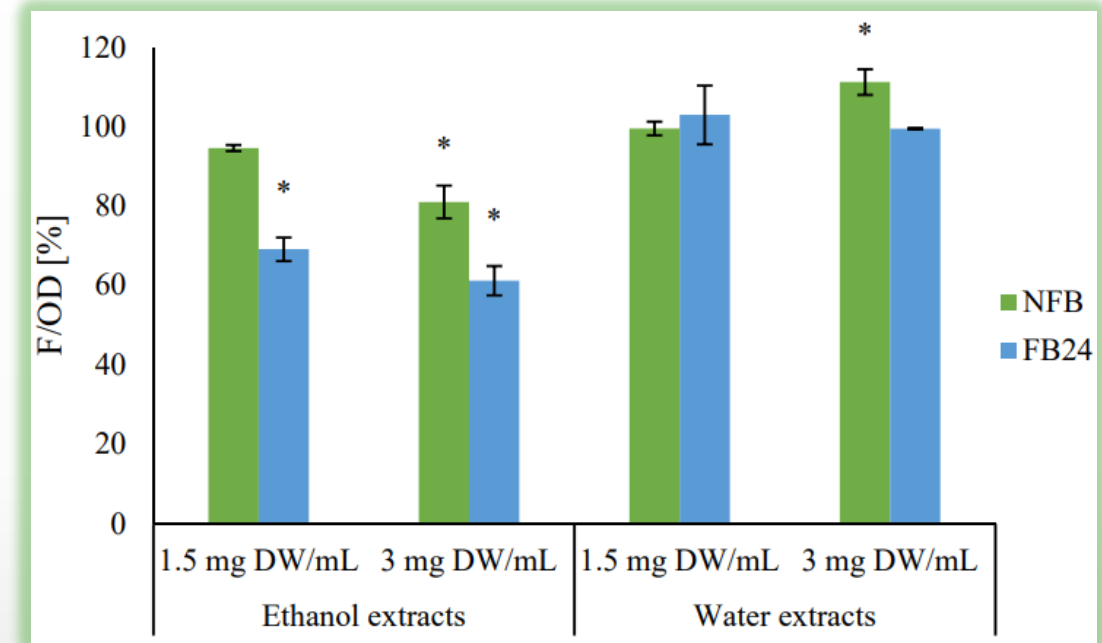
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## C: Yeast – cellular level

### Cell oxidation level:

- NFB and FB ethanol extracts (3 mg DW/mL):
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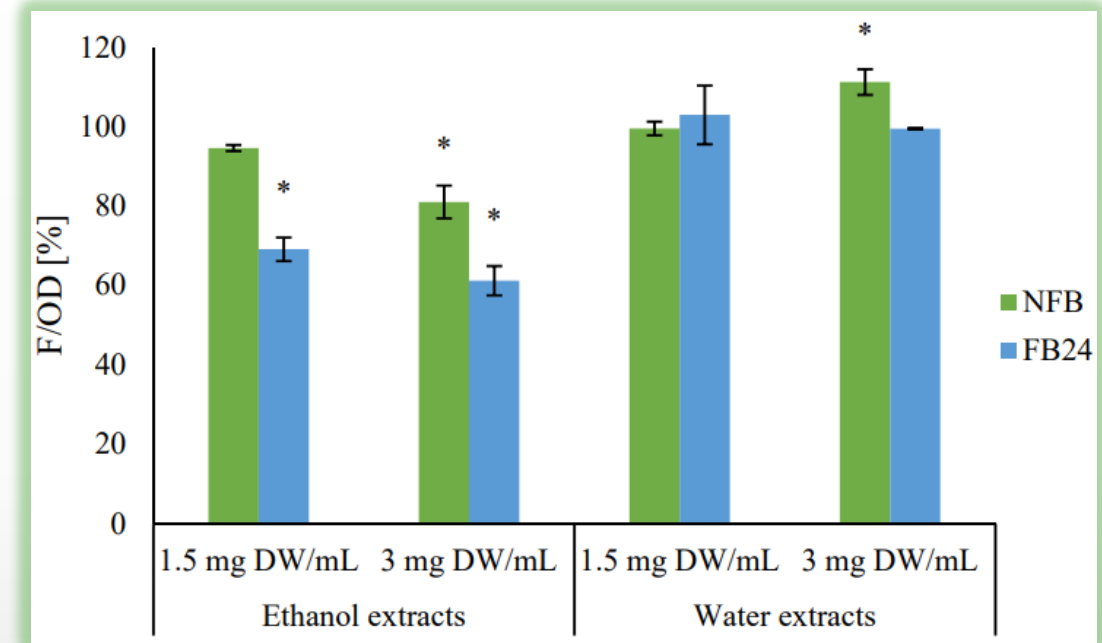
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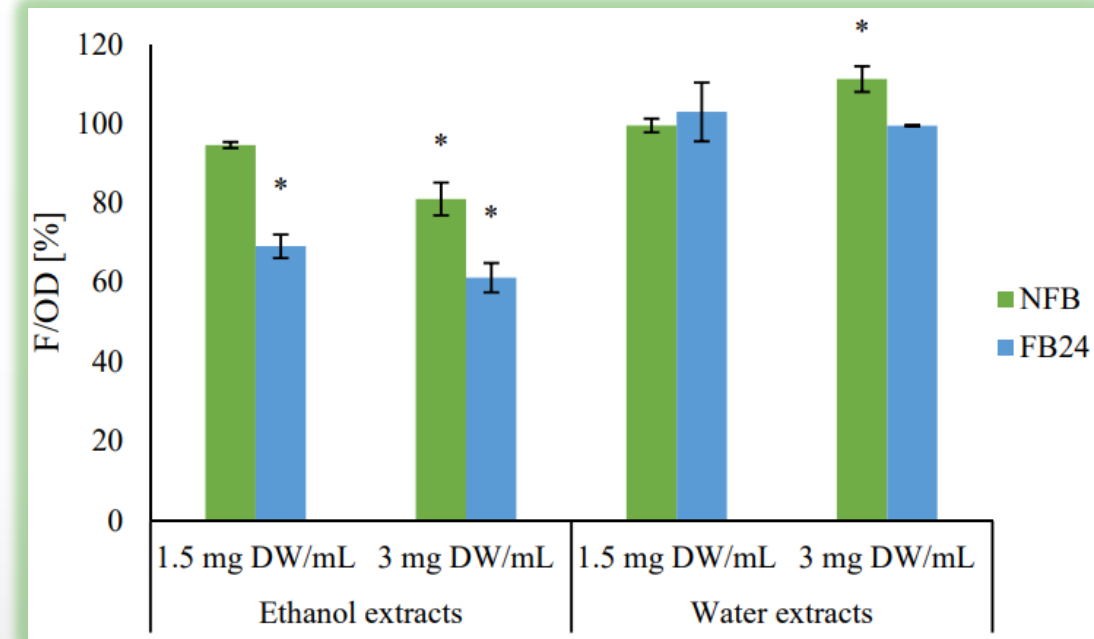
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Yeast cells	3.37 ± 0.54
Yeast cells + menadione (1 h)	13.8 ± 0.63
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### Lipid Peroxidation:

- Exposure to FB ethanol extract:

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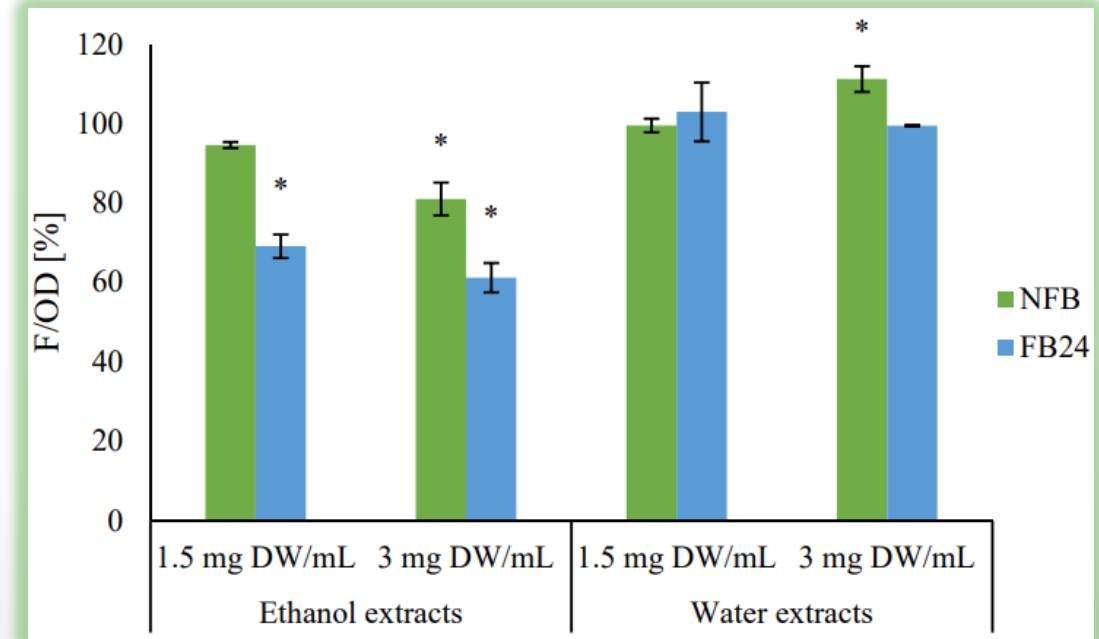
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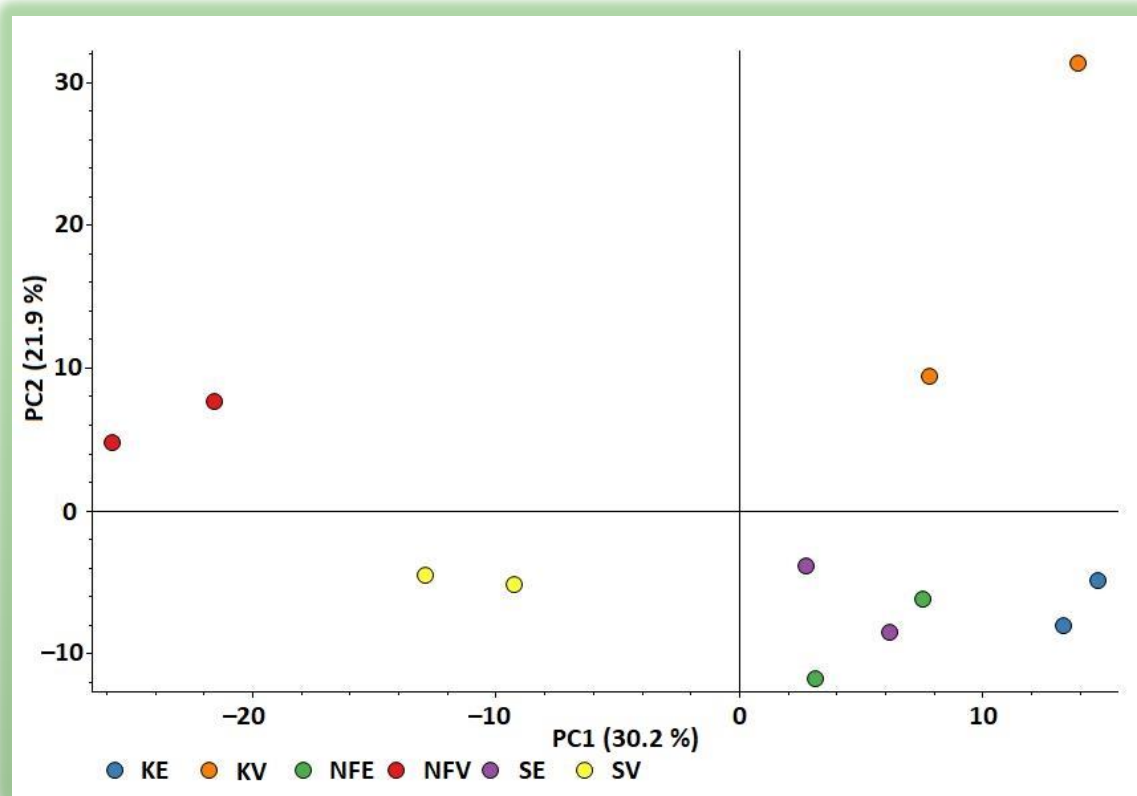
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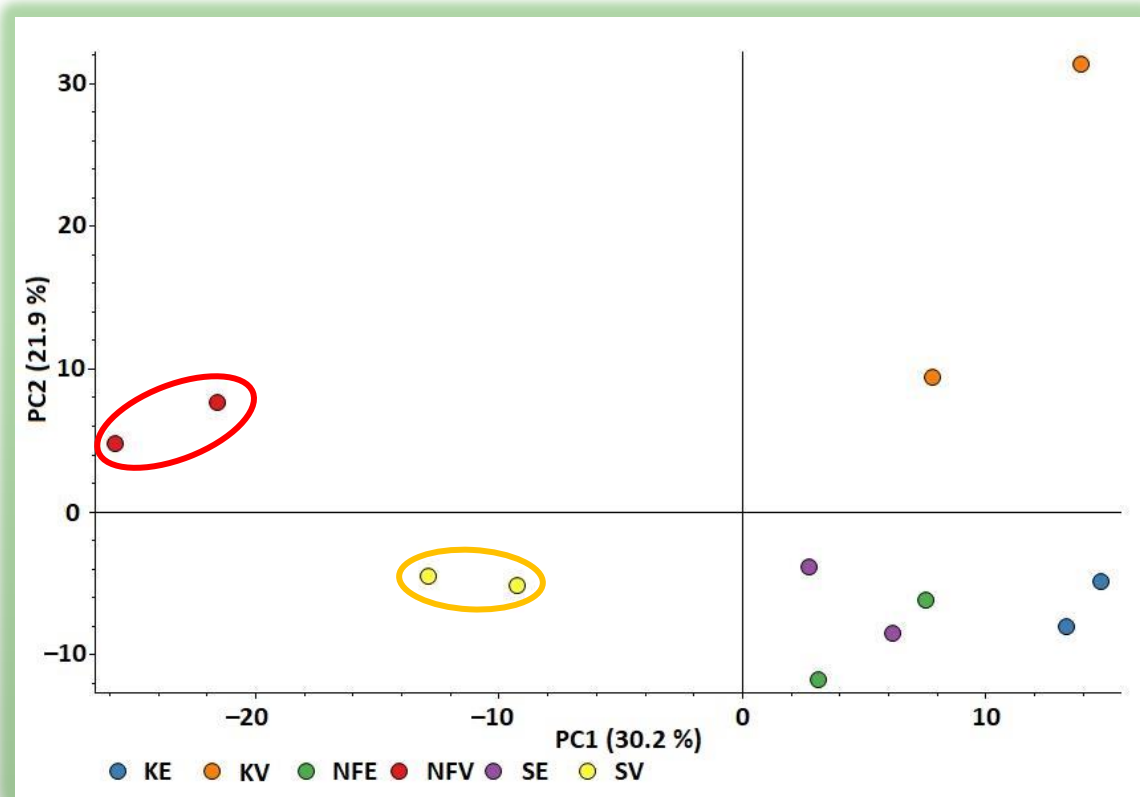
- Exposure to FB ethanol extract:

*Decreased oxidative damage*

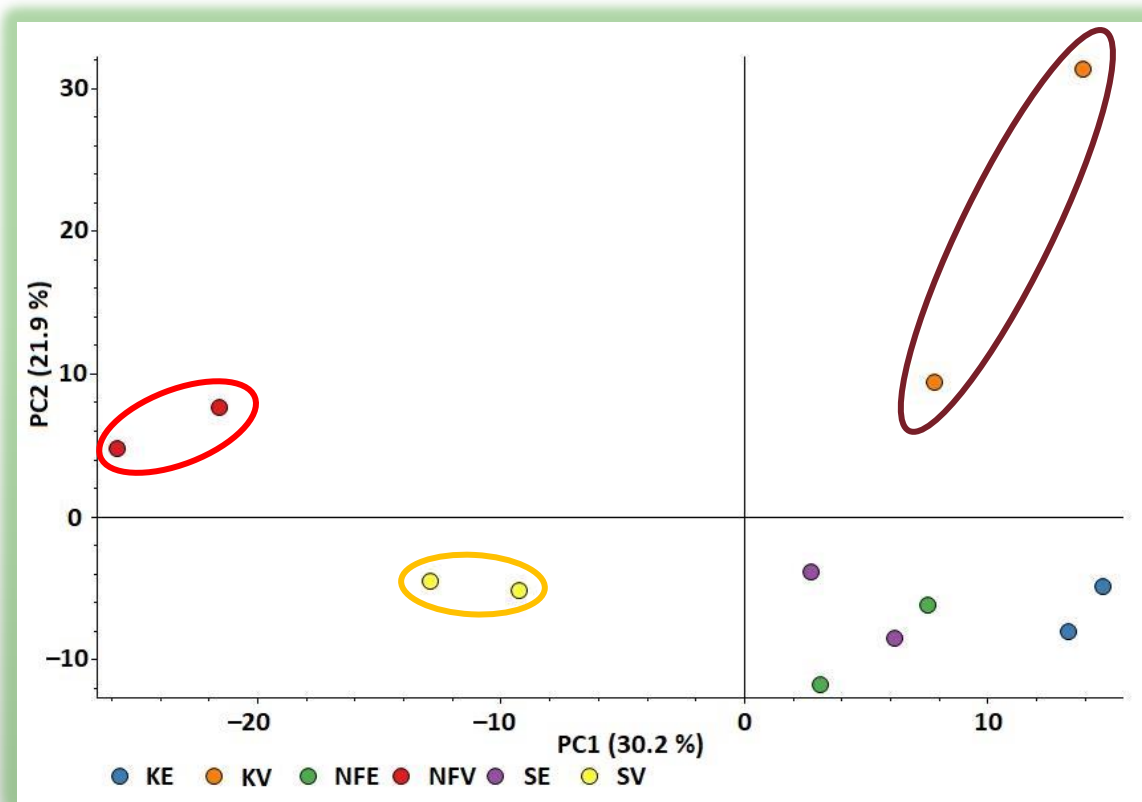
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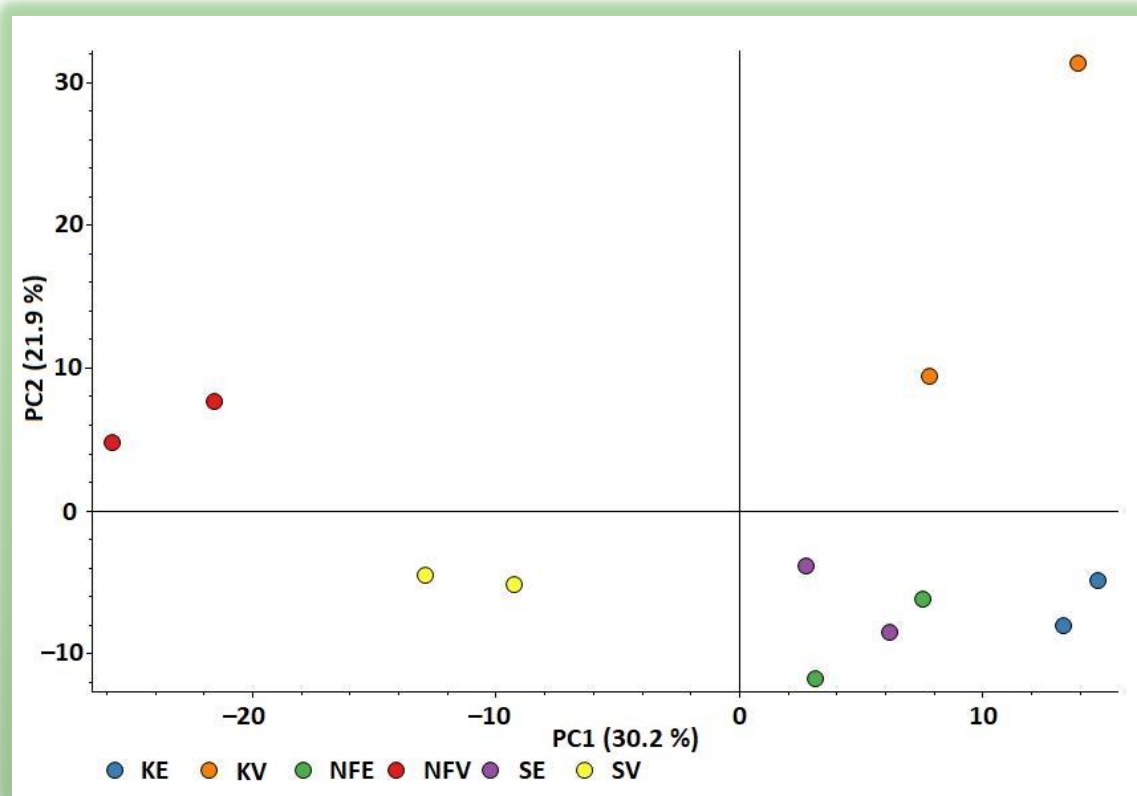
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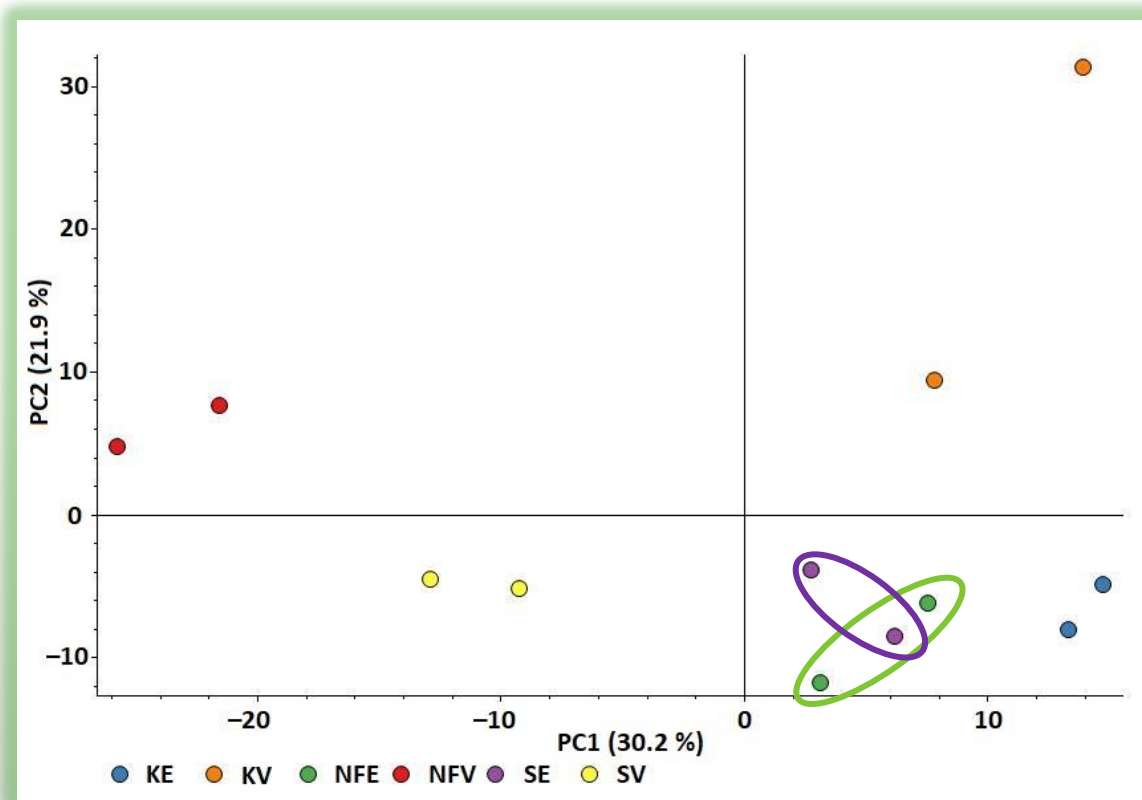
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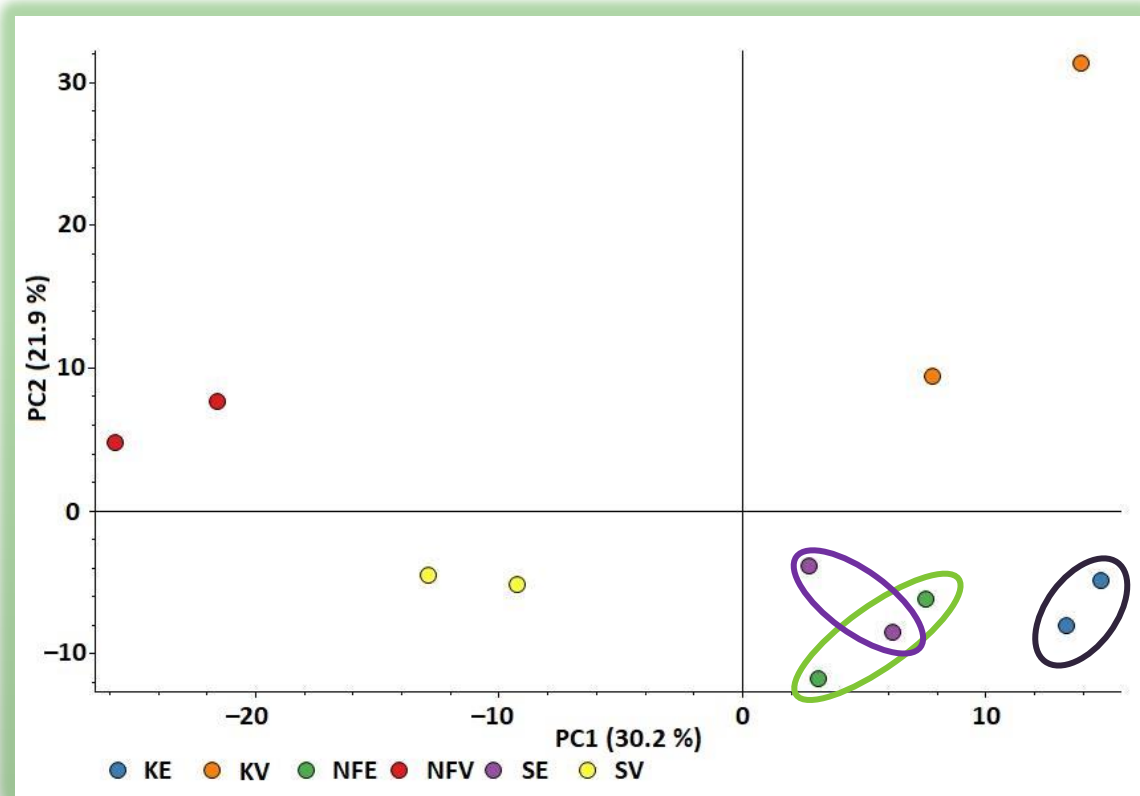


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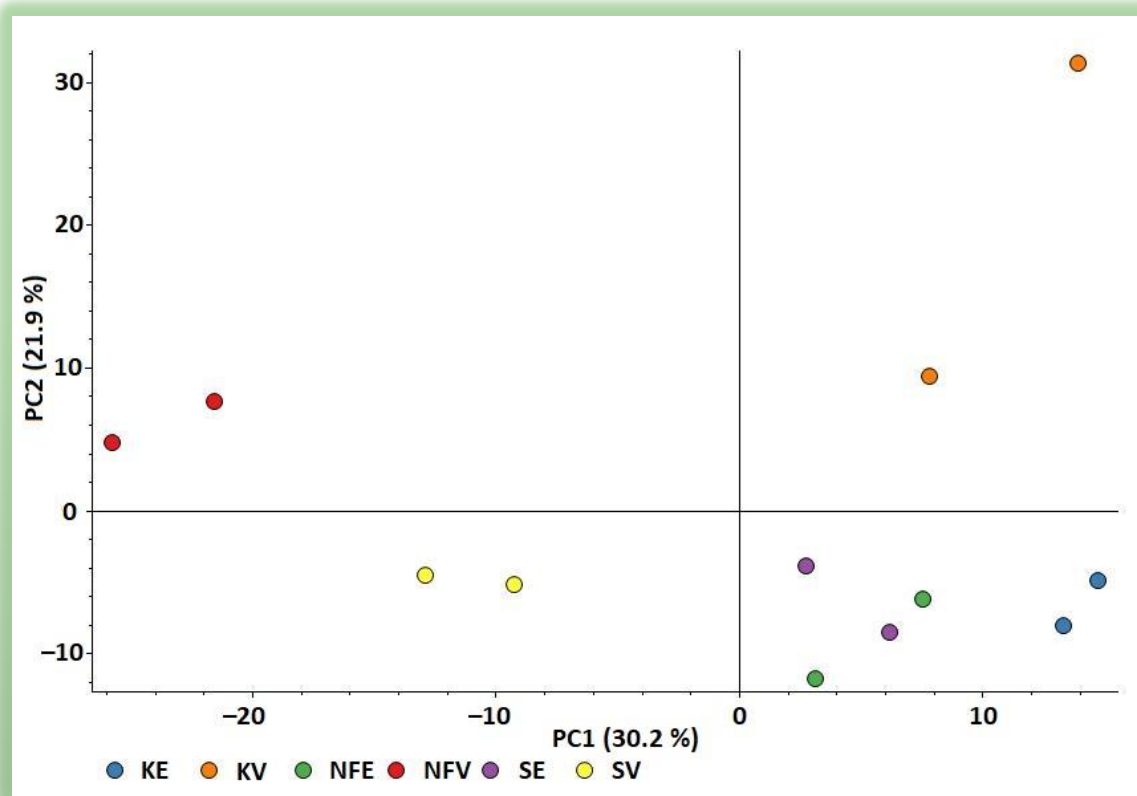




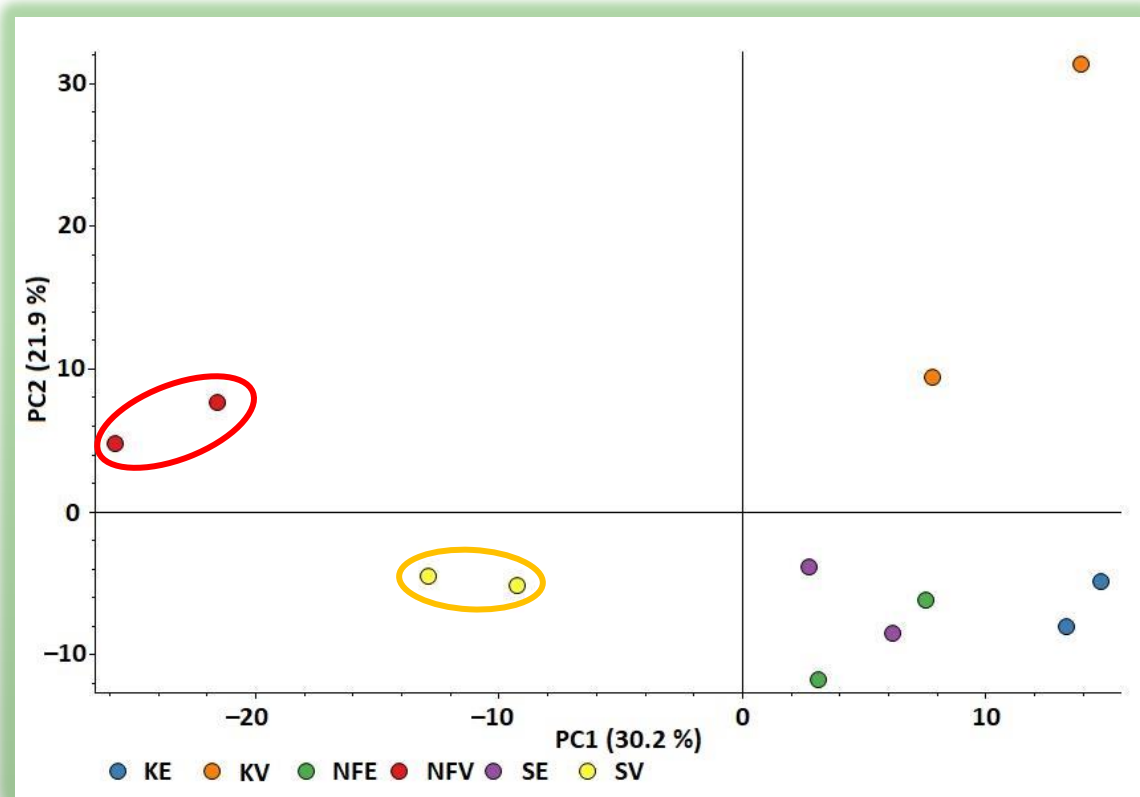
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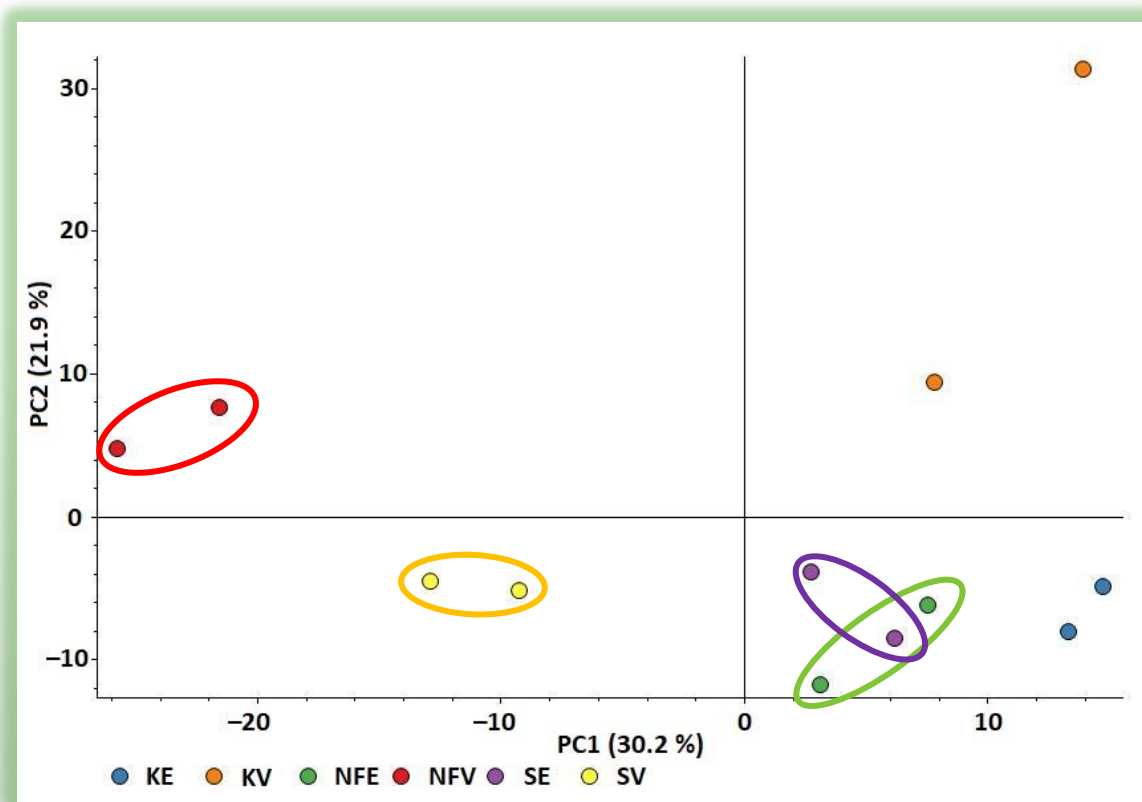
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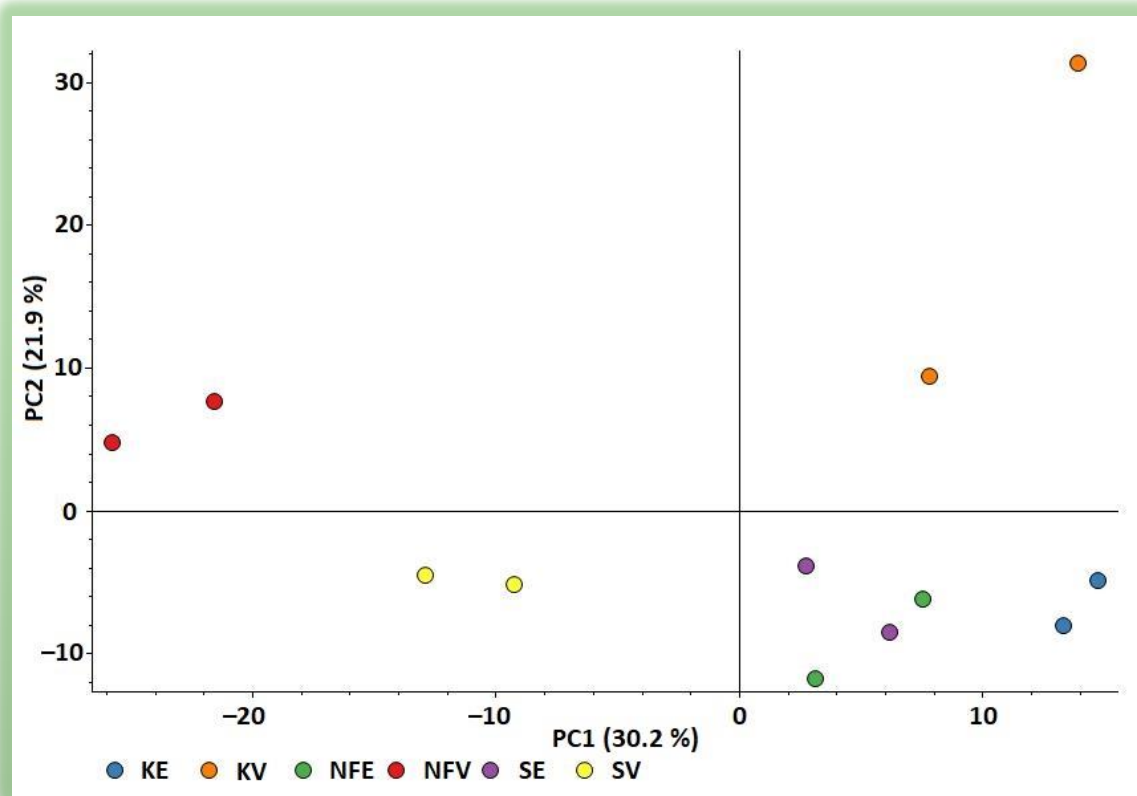
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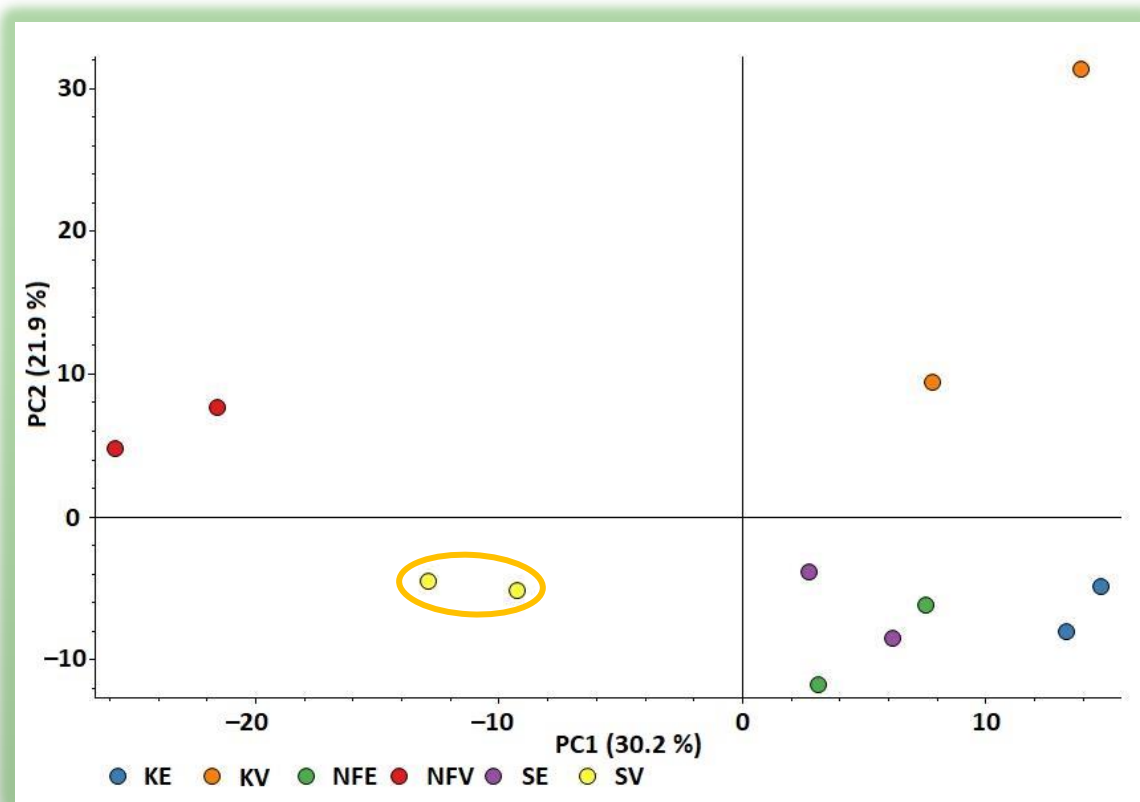
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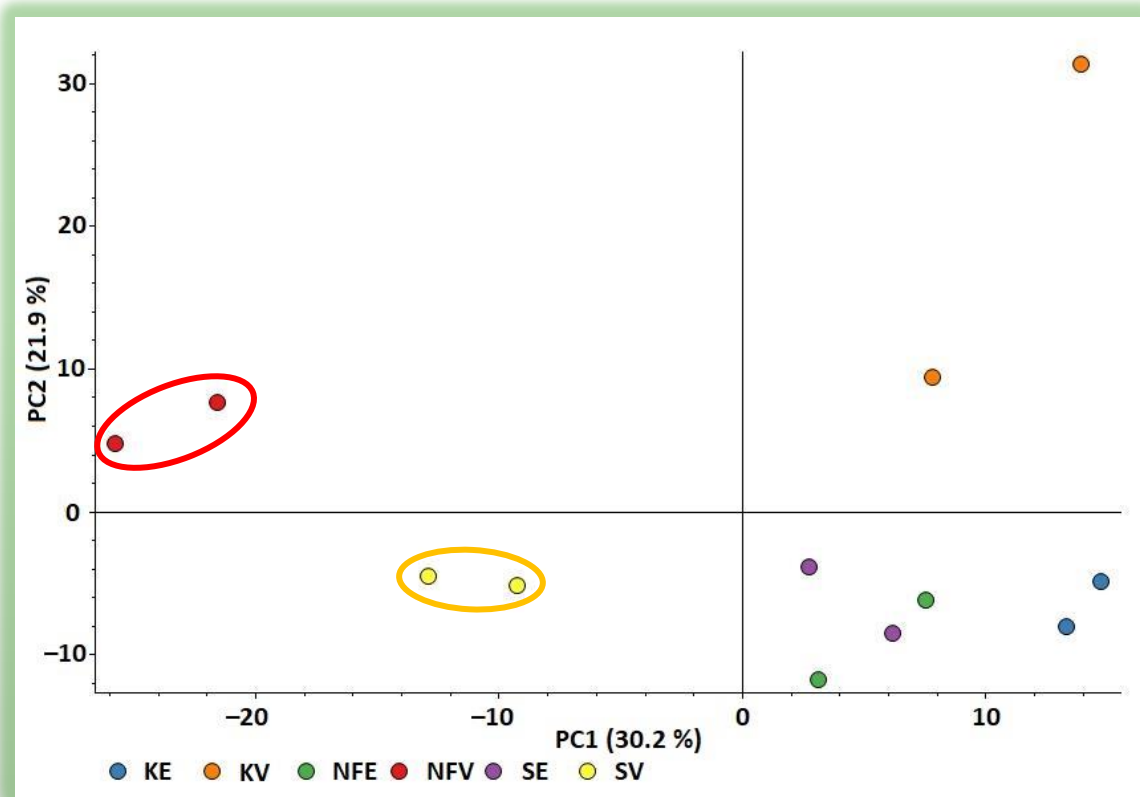
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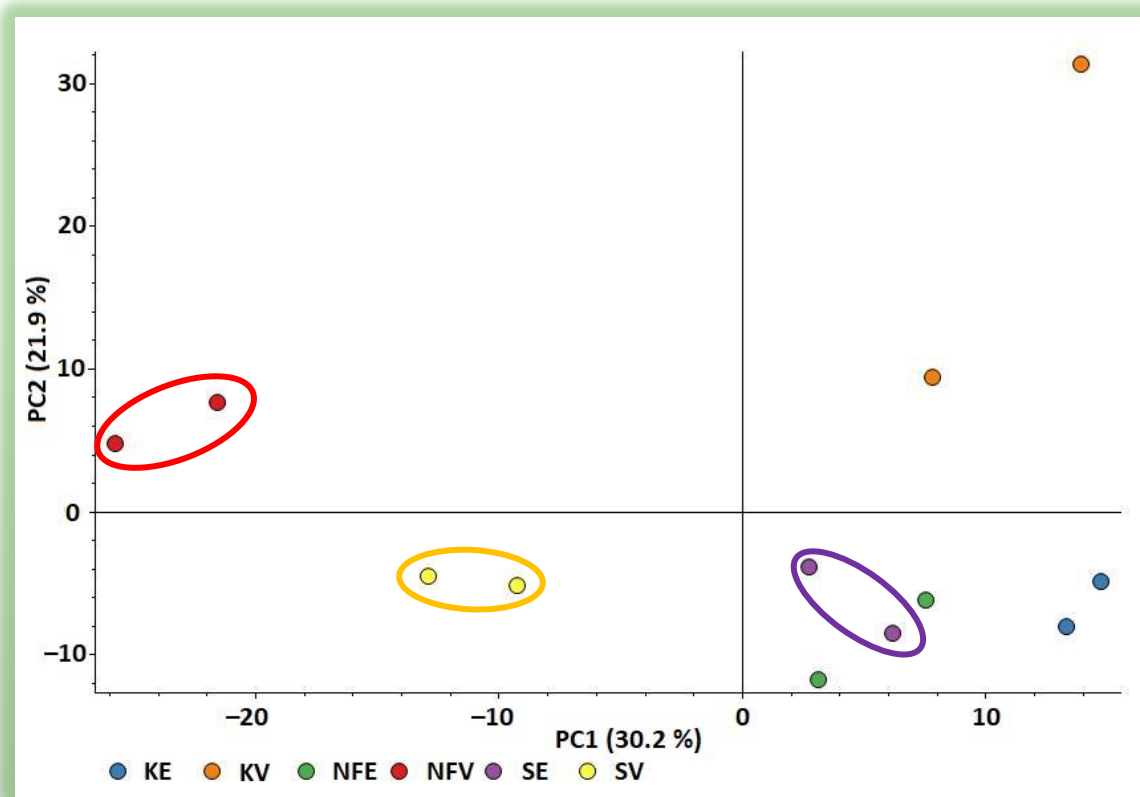
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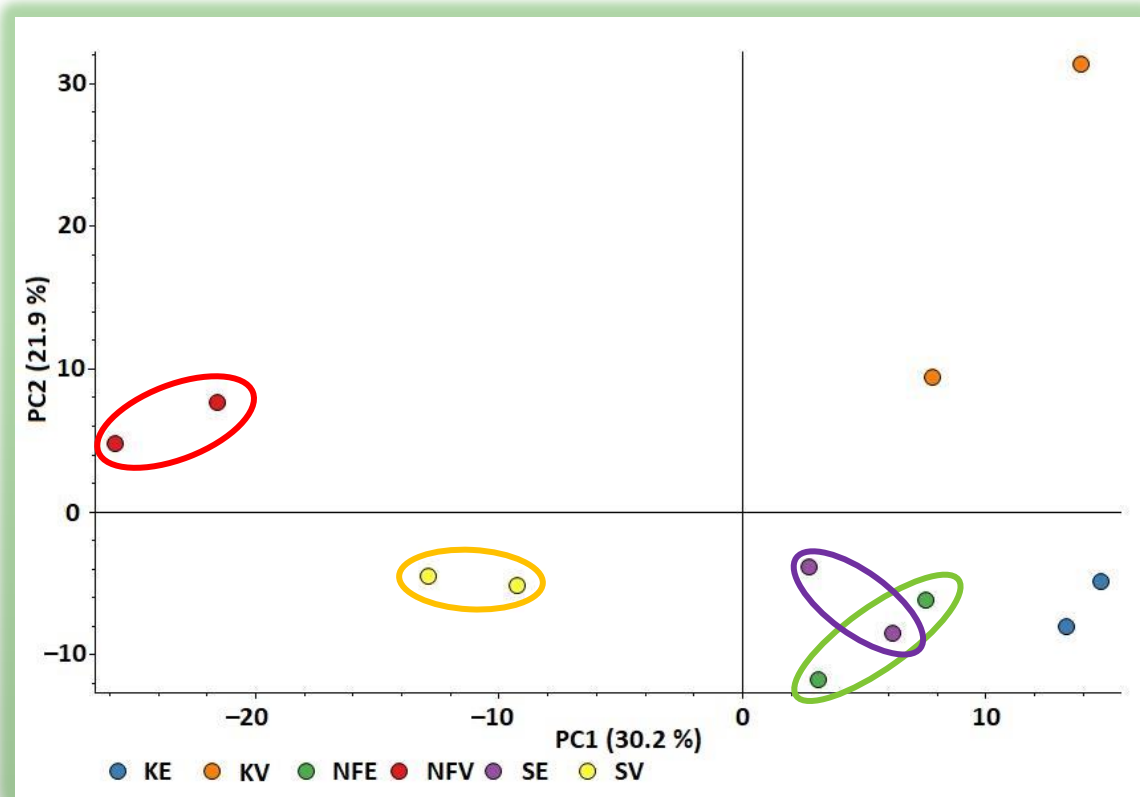


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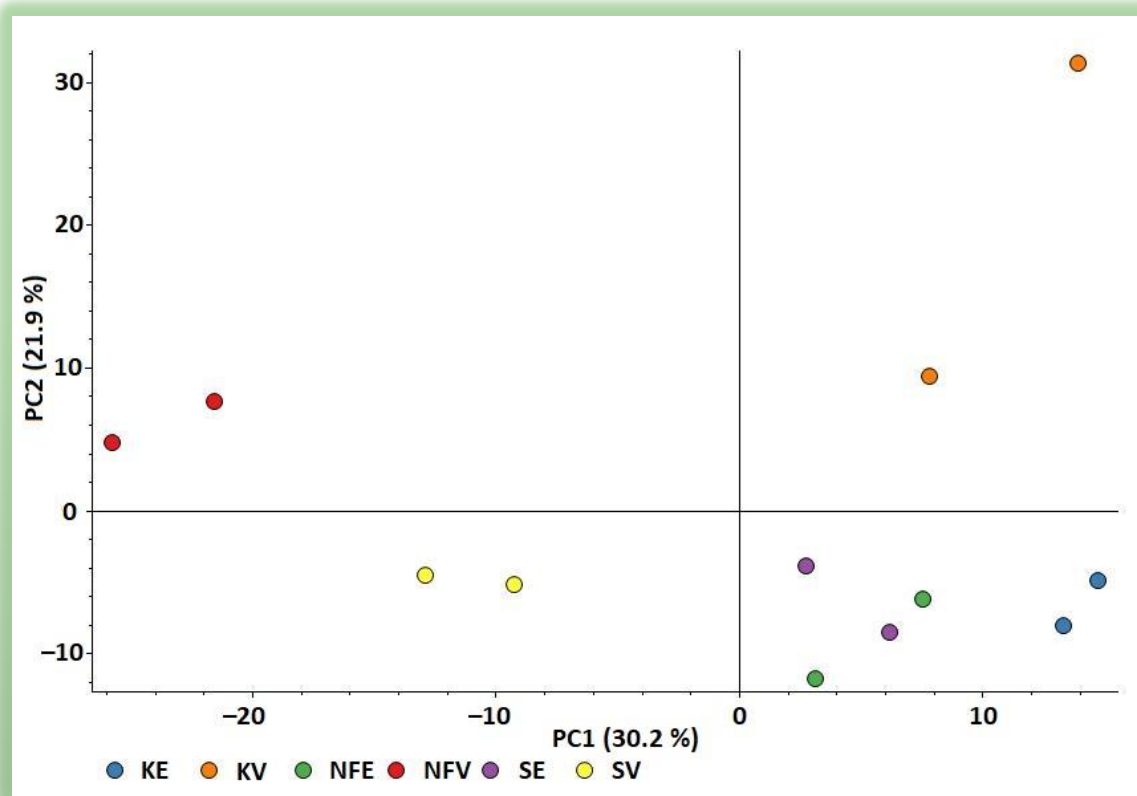




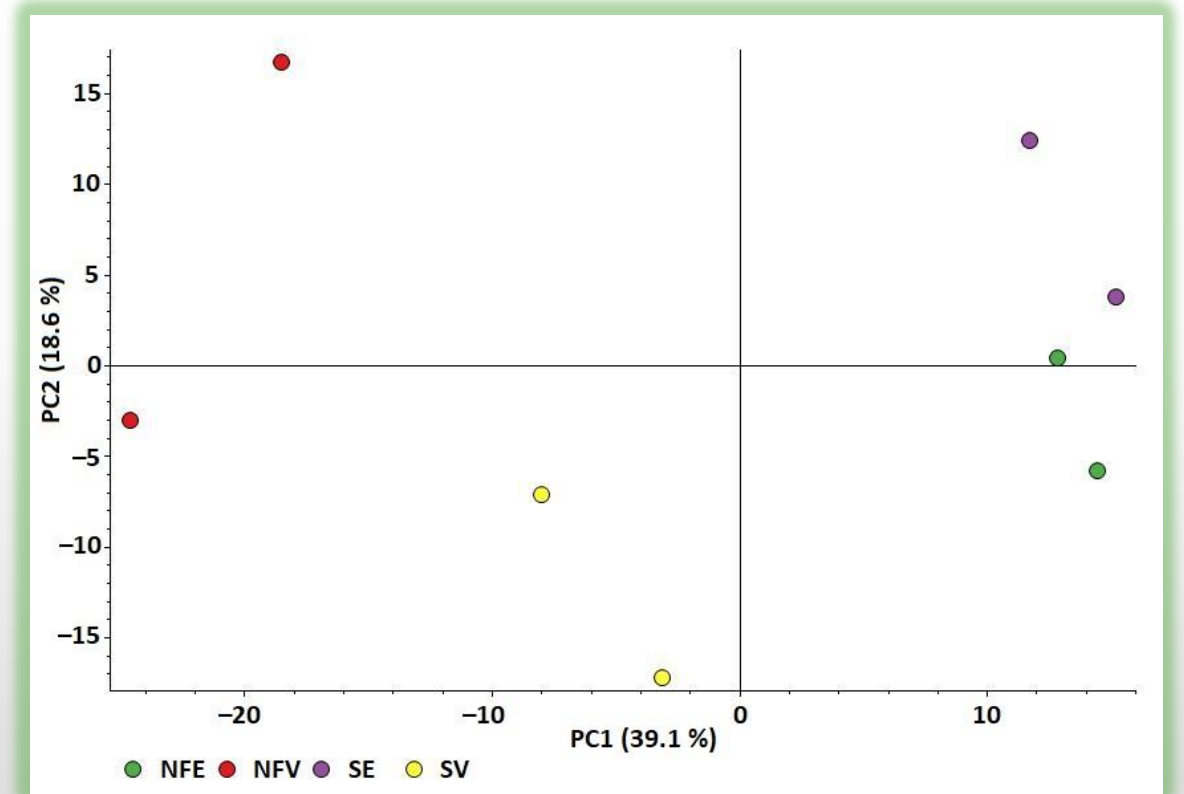
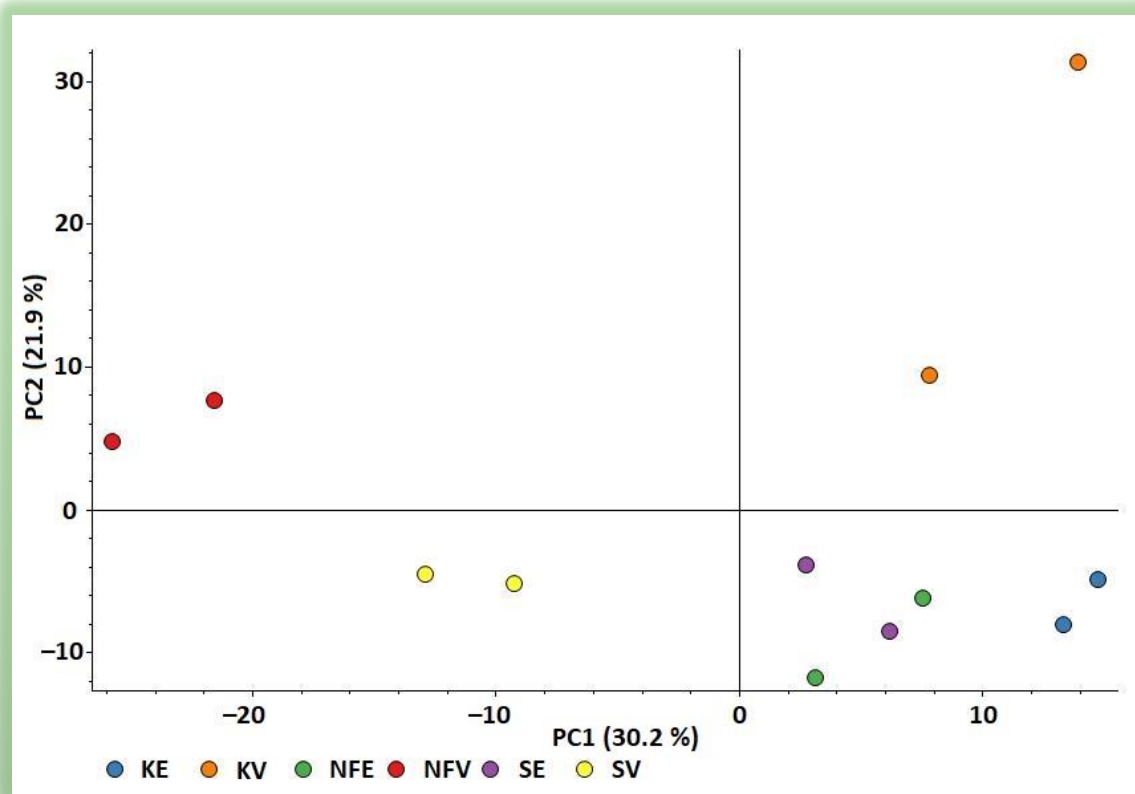
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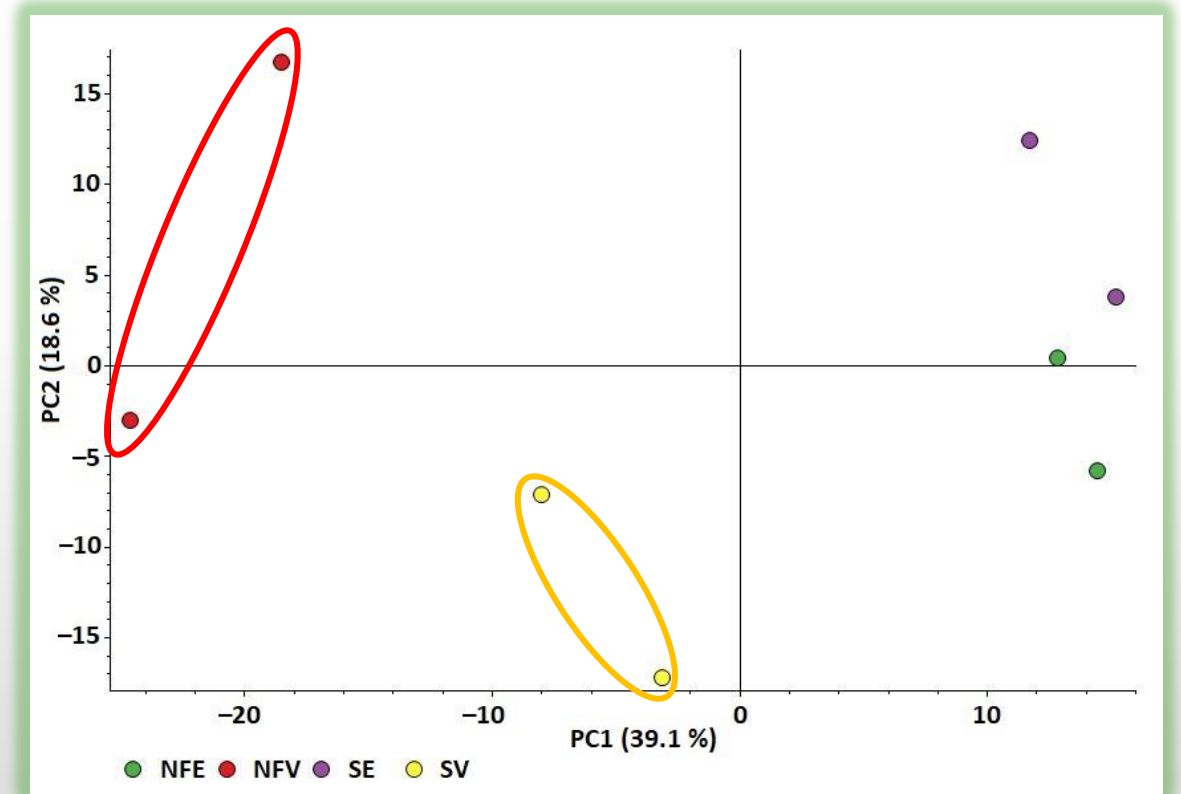
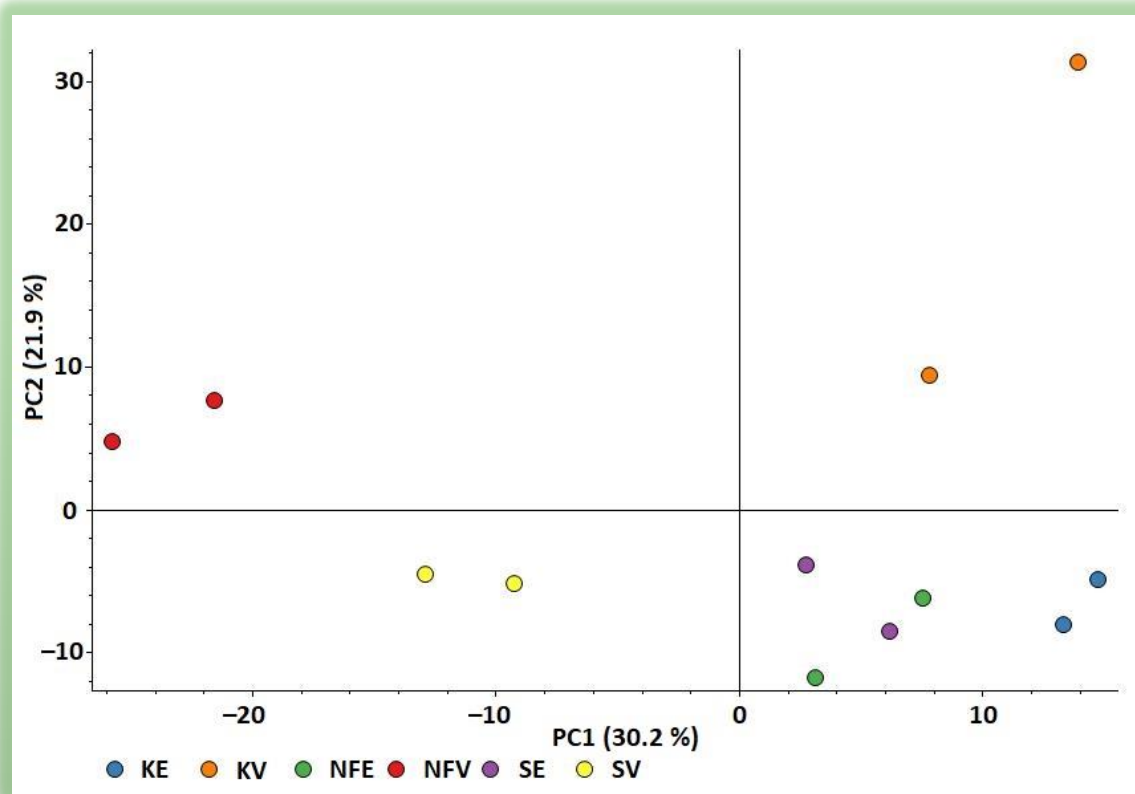
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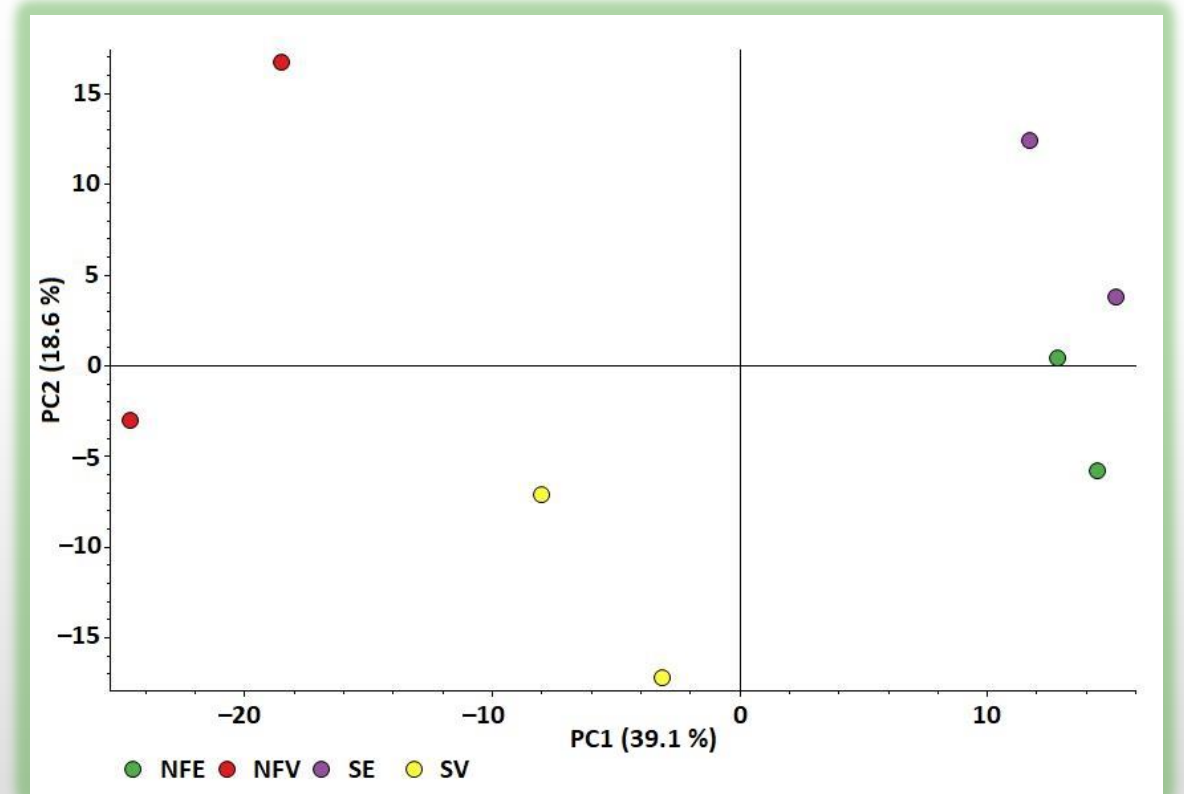
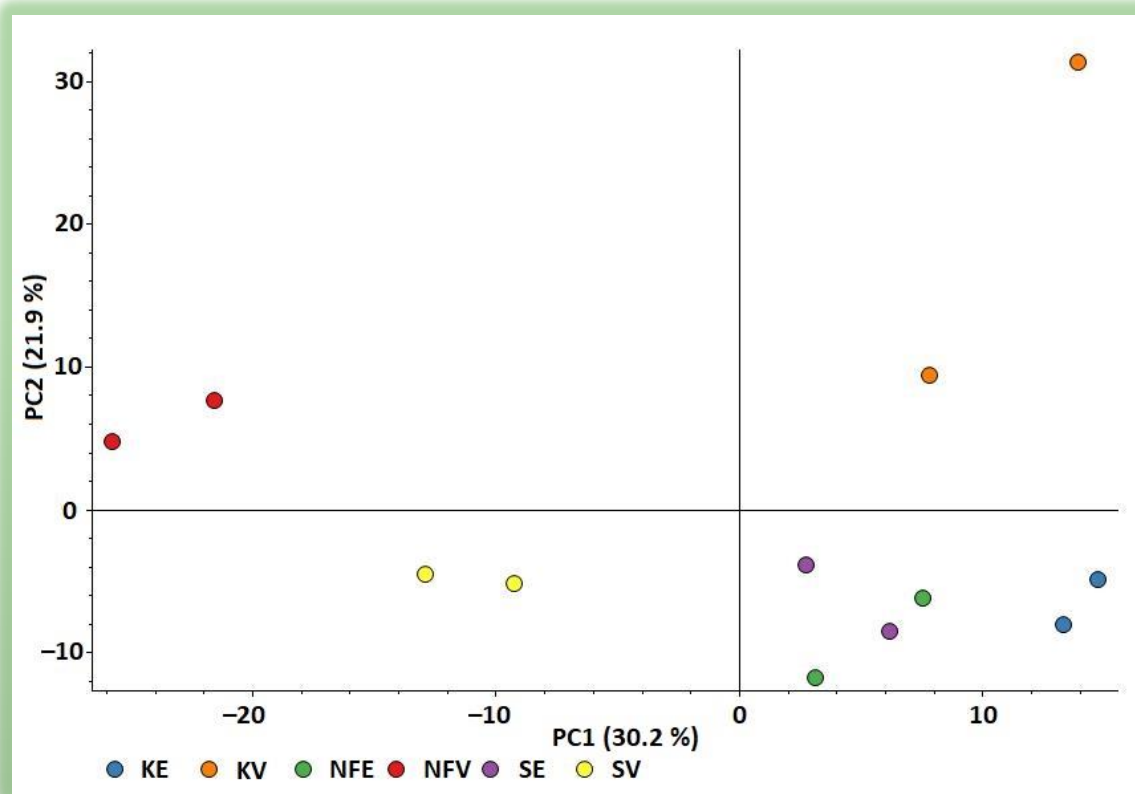
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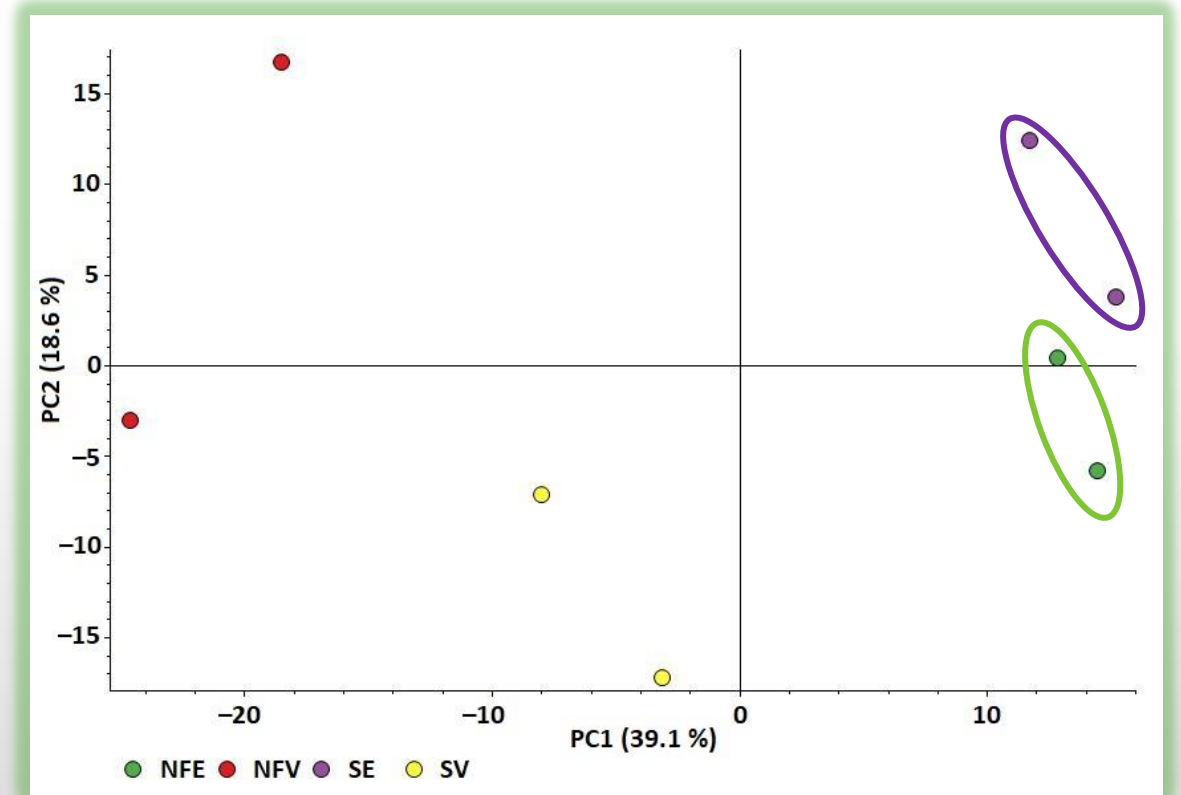
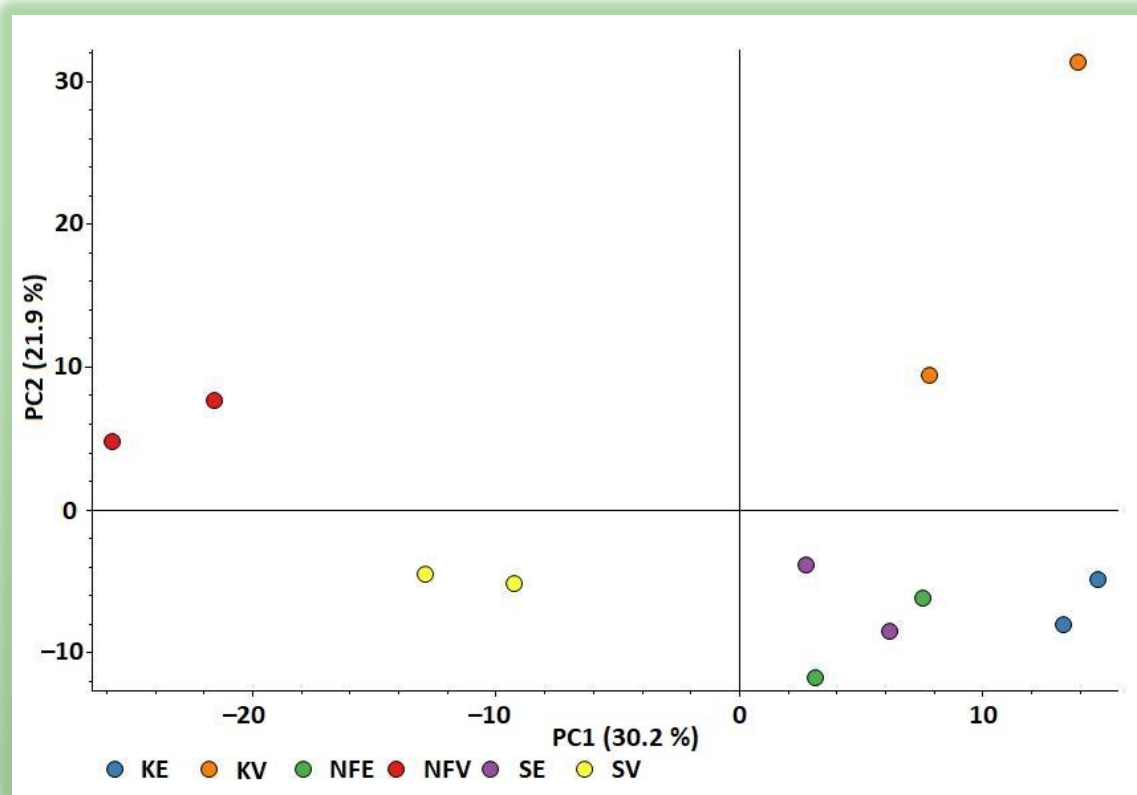
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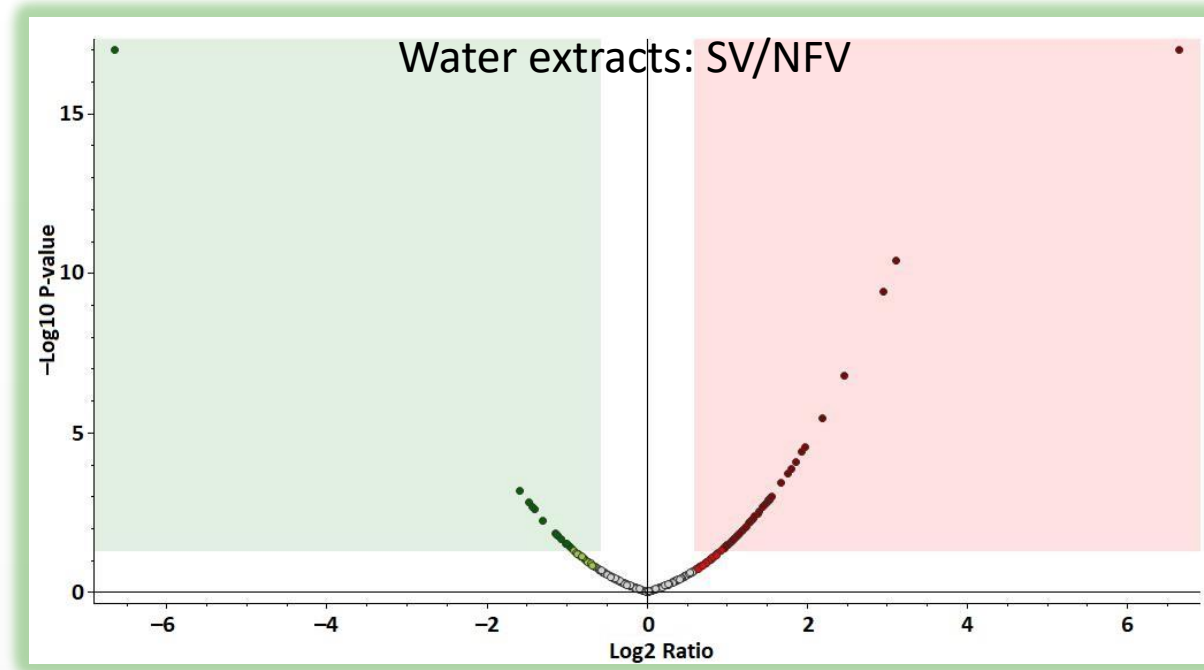
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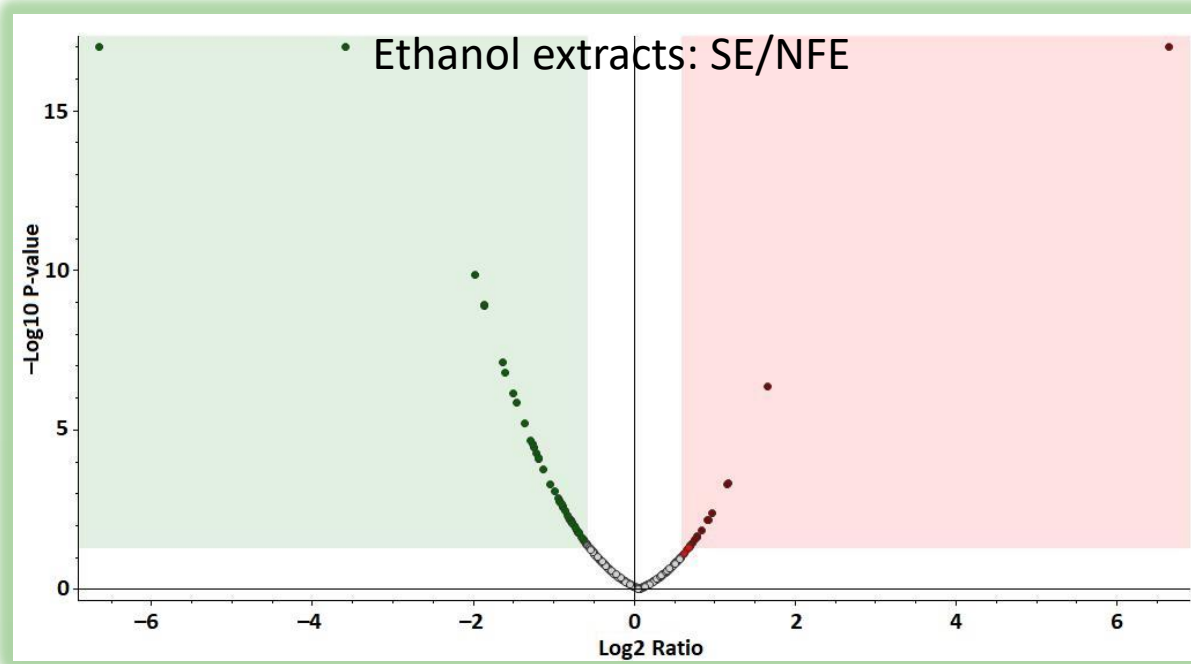
# C: Yeast proteome level



## Water extracts: SV/NFV

Proteins	Increase	Decline	Process	Why?
Gnd2		+	Glutathione metabolism	Higher cellular oxidative stress
Rpl14a, Rpl13b	+		Autophagy	Degradation of intracellular constituents
Rvb1, Gua1	+		DNA damage & repair, Purine biosynthesis	Need for nucleotide base synthesis
Pho3	+		Riboflavin metabolism	Antioxidative action to combat ROS

# C: Yeast proteome level



## Ethanol extracts: SE/NFE

Proteins	Increase	Decline	Process	Why?
Gnd2	+		Glutathione metabolism	Lower cellular oxidative stress
Gsh2		+	Glutathione biosynthesis	Lower need for glutathion
Rpl14a, Cue5		+	Autophagy	Reduced cell components' damage
Nas2, Nas6, Cub1		+	Proteasome function	Reduced protein damage
Cub1, Gua1, Ade13, Ura6		+	DNA repair/ purine, pyrimidine biosynthesis	Less DNA damage/need for nucleotides
Rib5		+	Riboflavin biosynthesis	Lower oxidative stress exposure



# Conclusions and Acknowledgements

Significant separation of:



No. 1000-17-0106  
Proj.no. J4-1773,  
P1-0143 and P4-0116



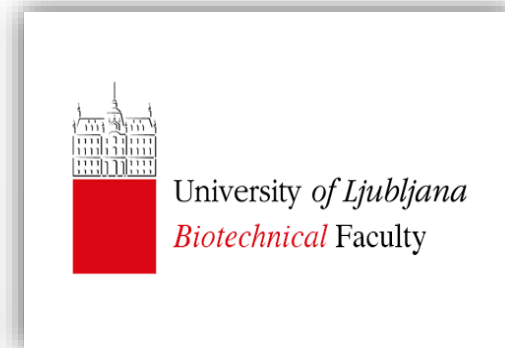
# Conclusions and Acknowledgements

## Significant separation of:

- Yeast treated with fermented/non-fermented *Spirulina*
- Yeast treated with ethanol/water extracts



No. 1000-17-0106  
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**Safety  
Quality  
Traceability**

# Conclusions and Acknowledgements

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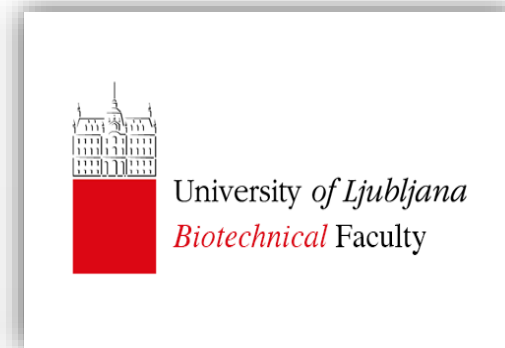
- Yeast treated with fermented/non-fermented *Spirulina*
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## Greater antioxidant efficiency of:

- Ethanol vs. water extracts in F vs. NF *Spirulina*



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# Conclusions and Acknowledgements

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- Yeast treated with fermented/non-fermented *Spirulina*
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## Greater antioxidant efficiency of:

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## Essential role of fermentation:

- Lowering of cell stress response related proteins expression.



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