Master of Science in Marine Biological Resources

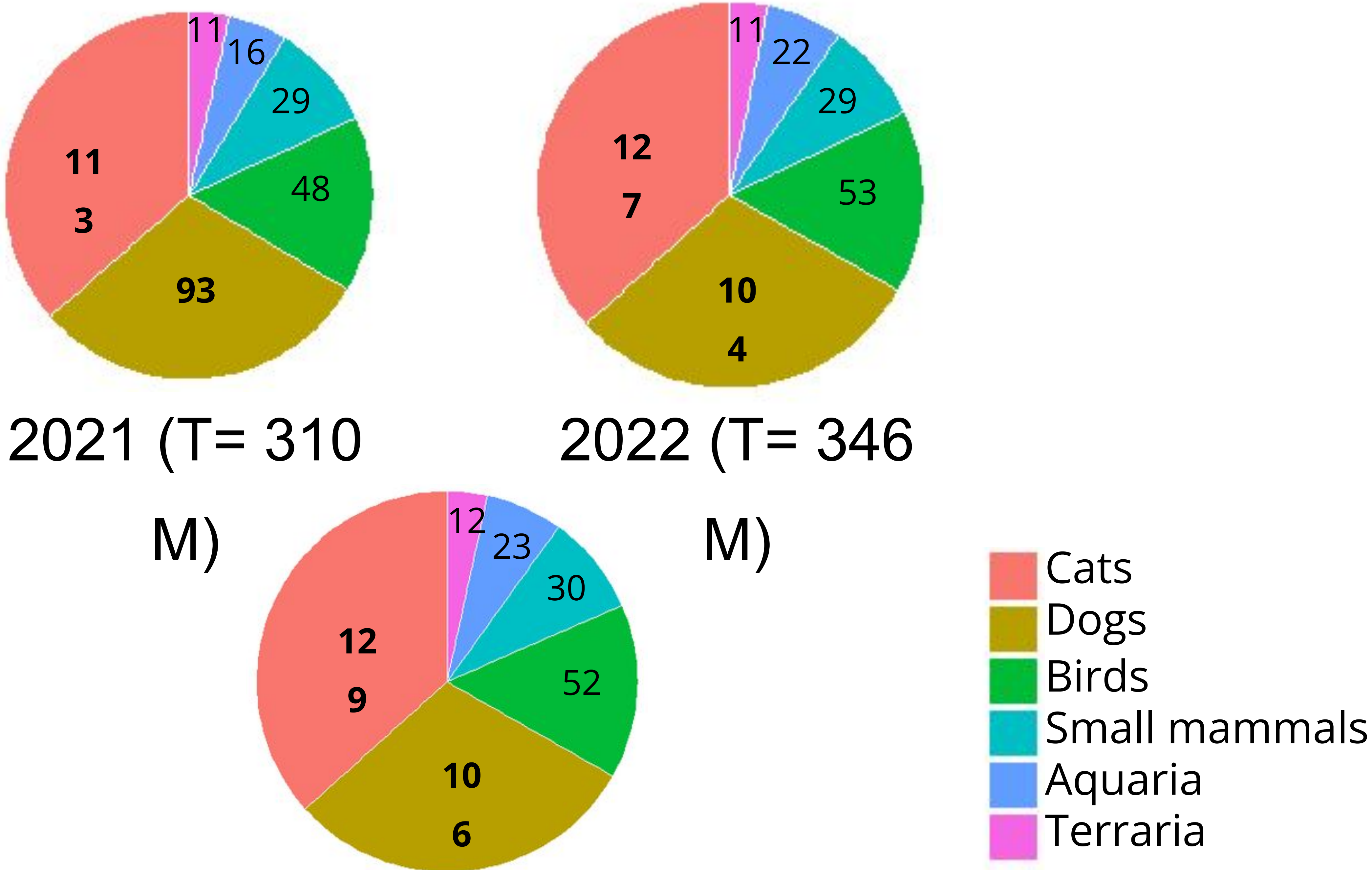
# Analysis and utilization of Ulva rigida, Palmaria palmata, and Fucus vesiculosus for potential dog and cat snacks

Author: Francisco Nomdedeu Martinez

Master thesis - July 2024

Supervisor: Luísa Margarida Batista Custodio

## Europe




2021 (T= 310 M)

2022 (T= 346 M)

2023 (T= 352

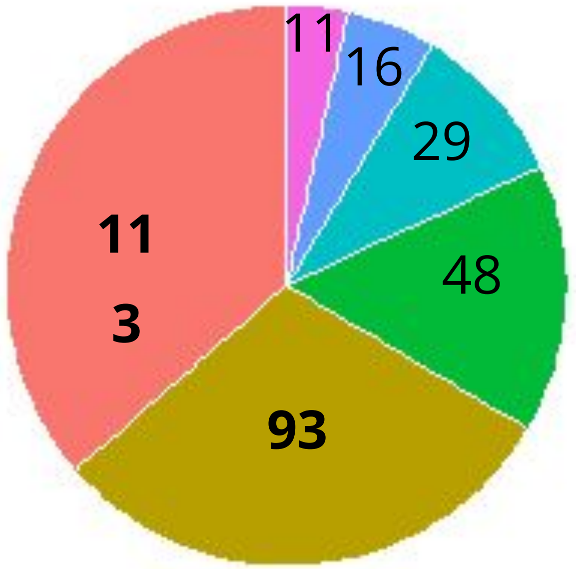
Units in millions of individuals (M)



FEDIAF, 2022; FEDIAF, 2023; FEDIAF, 2024

# INTRODUCTION:

## Europe

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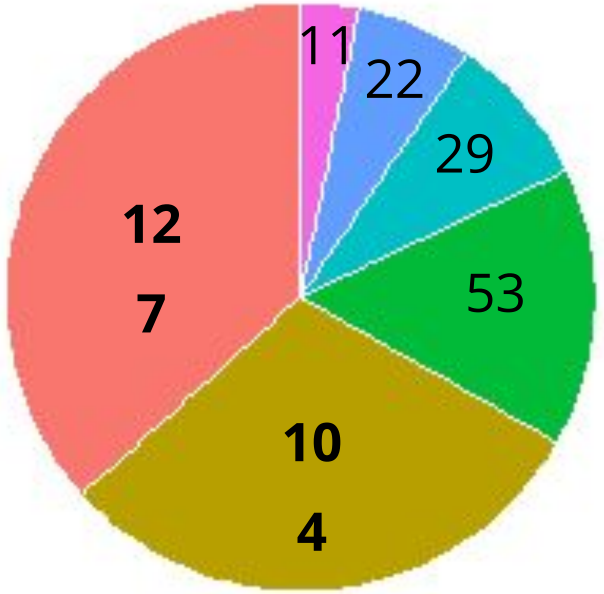


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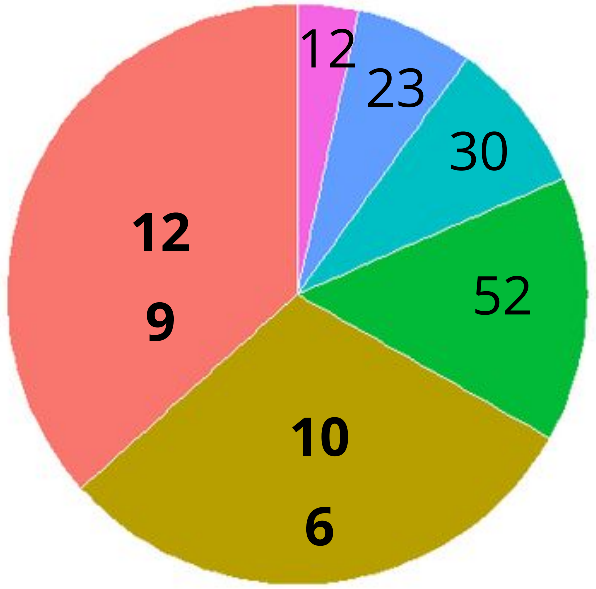
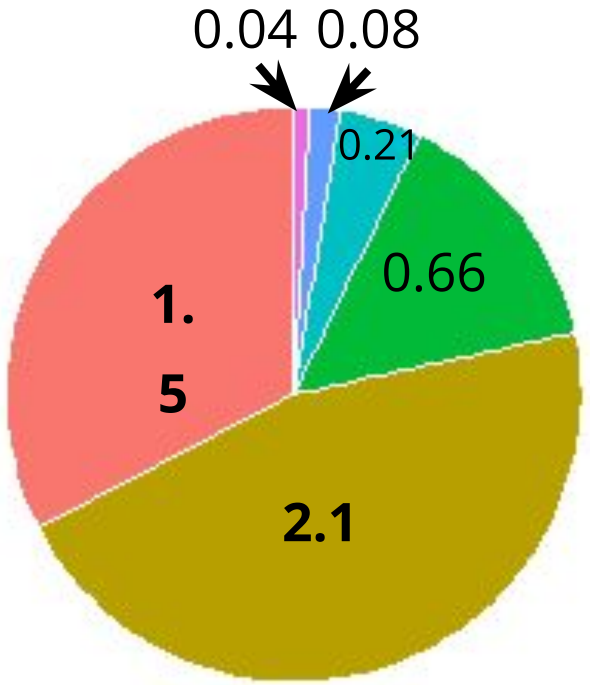


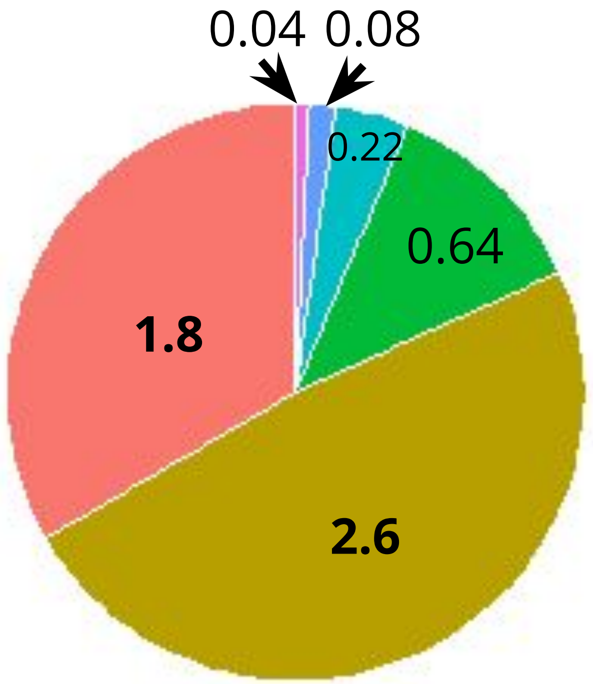

2023 (T= 352


Units in millions of individuals (M)

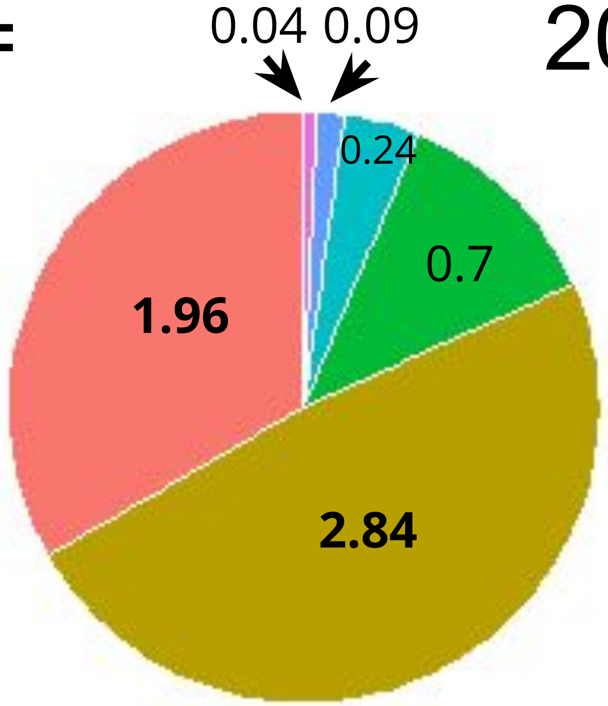
## Portugal




2021 (T= 4.59M)




2022 (T= 5.38 M)



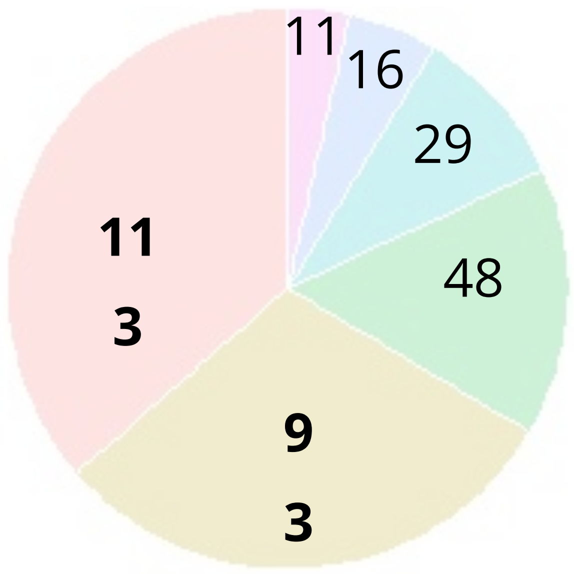

2023 (T= 5.87



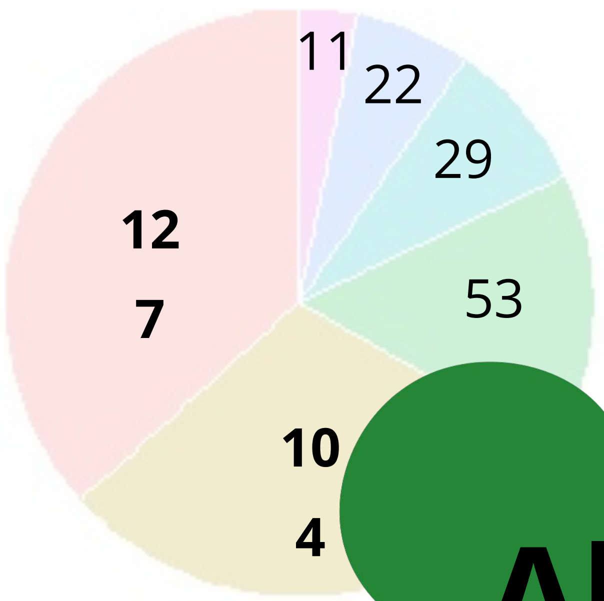
FEDIAF, 2022; FEDIAF, 2023; FEDIAF, 2024

## INTRODUCTION:

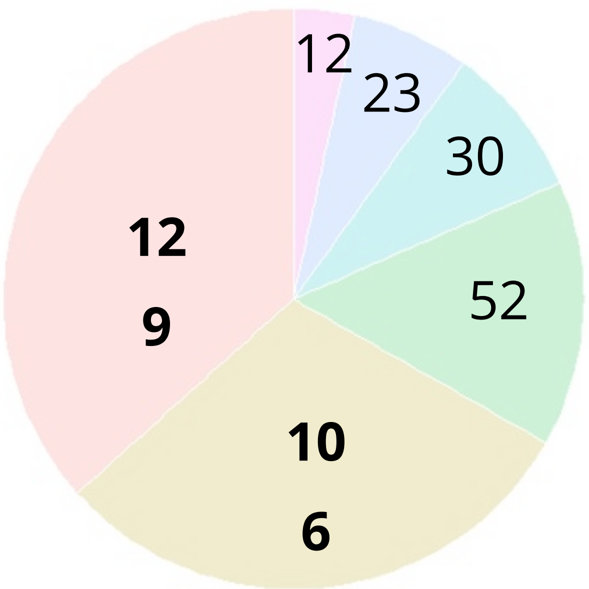
### Europe




2021 (T= 310 M)




2022 (T= M)



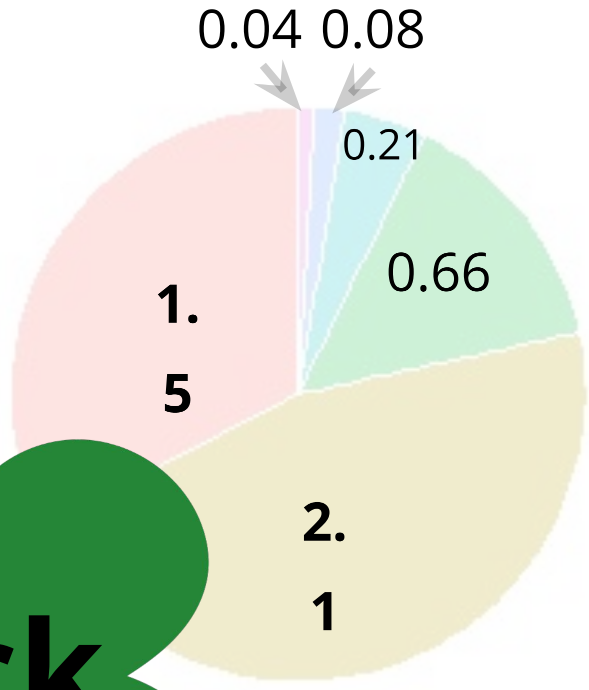

2023 (T= 352

**Algae snack**

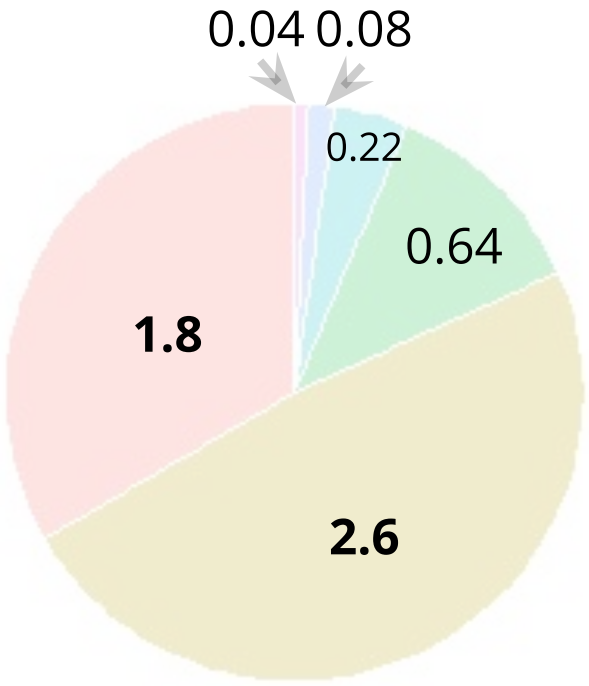
- Cat
- Dogs
- Birds
- Small mammals
- Aquaria
- Terraria

Units in millions of individuals (M)

### Portugal




21 (T= 4.59M)

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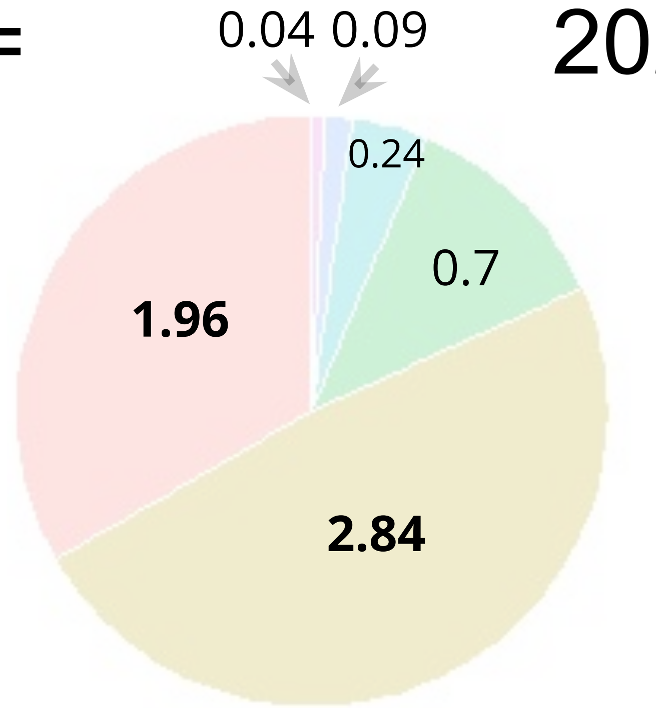


2023 (T= 5.87



FEDIAF, 2022; FEDIAF, 2023; FEDIAF, 2024

# INTRODUCTION:

PET

**Algae snack**

Green algae (Chlorophyta):

Ulva rigida

- Antioxidant power
- Nutricional composition:
  - Proteins
  - Lipids
  - Minerals (Calcium, Iron, Magnesium, Potassium and Sodium)
  - Carotenoids
  - Vitamins

(Makkar et al., 2016)

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Red algae (Rhodophyta):

Palmaria palmata

- Nutricional composition:
    - Proteins
    - Carbohydrates
    - Lipids
    - Minerals (Calcium, Iron, Magnesium, Potassium, Iodine and Sodium)
    - Fatty acids
    - Vitamins



(De Clerck et al., 2012; Makkar et al., 2016)

# INTRODUCTION:

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  - Fatty acids
  - Vitamins

Brown algae (Phaeophyta):

Fucus vesiculosus

- Mineral content
- Anti-inflamatory power

(De Clerck et al., 2012; Makkar et al., 2016; Balina et al., 2016; Obluchinskaya et al., 2022)

PET

# Nutritional and Bioactive Content of Pet Snacks

Algae snack

**Complementary Role**: Snacks help fill nutritional gaps in pets' diets. and offer a healthy and diverse feeding option.

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**KEY PARAMETERS**

PET

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**Antioxidant Power**

**KEY PARAMETERS**

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Algae snack

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**KEY PARAMETERS**

- **Antioxidant Power**
- **Enzymatic Inhibitory Properties**

INTRODUCTION:

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**KEY PARAMETERS**

- **Antioxidant Power**
- **Enzymatic Inhibitory Properties**
- **Anti-inflammatory Properties**
- **Protein, Sugar, Lipid and Mineral content**

INTRODUCTION:

PET

# Nutritional and Bioactive Content of Pet Snacks

Algae snack

**Complementary Role**: Snacks help fill nutritional gaps in pets' diets. and offer a healthy and diverse feeding option.

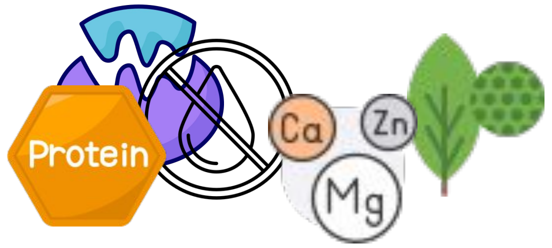
**KEY PARAMETERS**

- **Antioxidant Power**
- **Enzymatic Inhibitory Properties**
- **Anti-inflammatory Properties**
- **Protein, Sugar, Lipid and Mineral content**

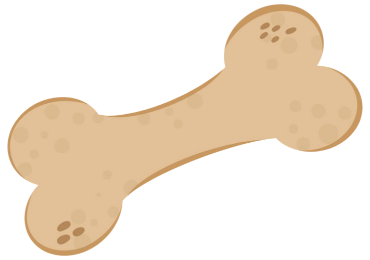
**Health Benefits**: Boosts immune system, Improves digestion and ensures balanced nutrition with essential nutrients.

## OBJECTIVES:

- Comprehensive **analysis** of the **bioproducts** derived from **algae**



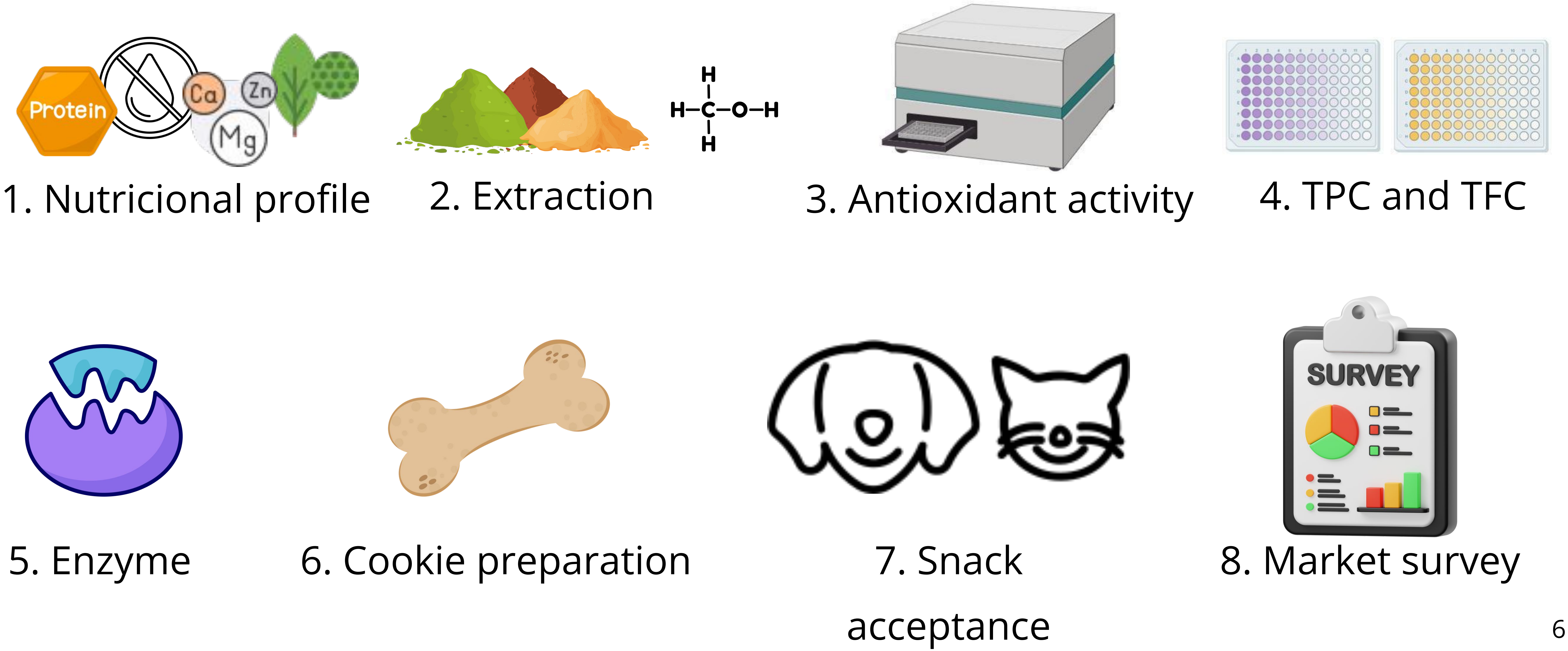

- Formulate and **produce pet biscuits** incorporating these **algae**



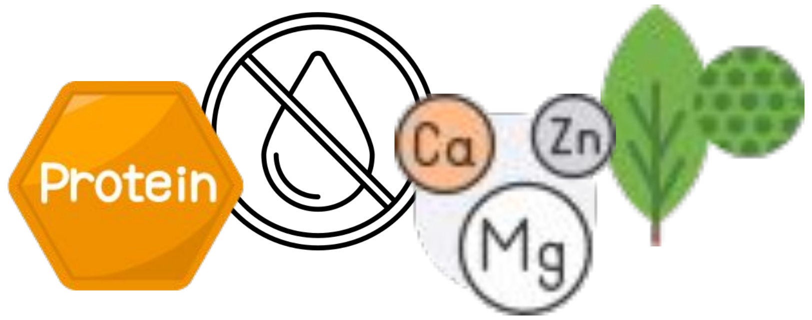
- Evaluation the **acceptance** among dogs and cats


- Consumers **survey**

# GRAPHICAL ABSTRACT:



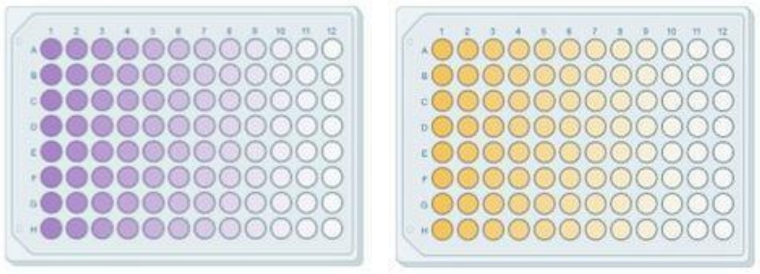
# GRAPHICAL ABSTRACT:




1. Nutricional profile

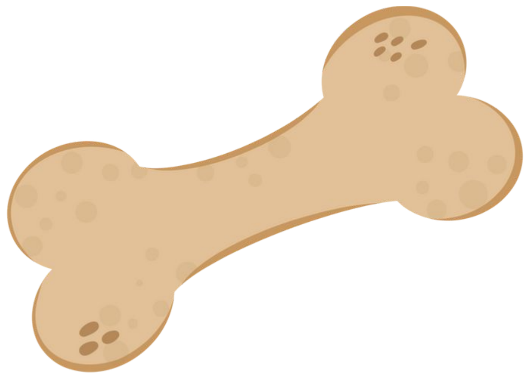

2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme



6. Cookie preparation

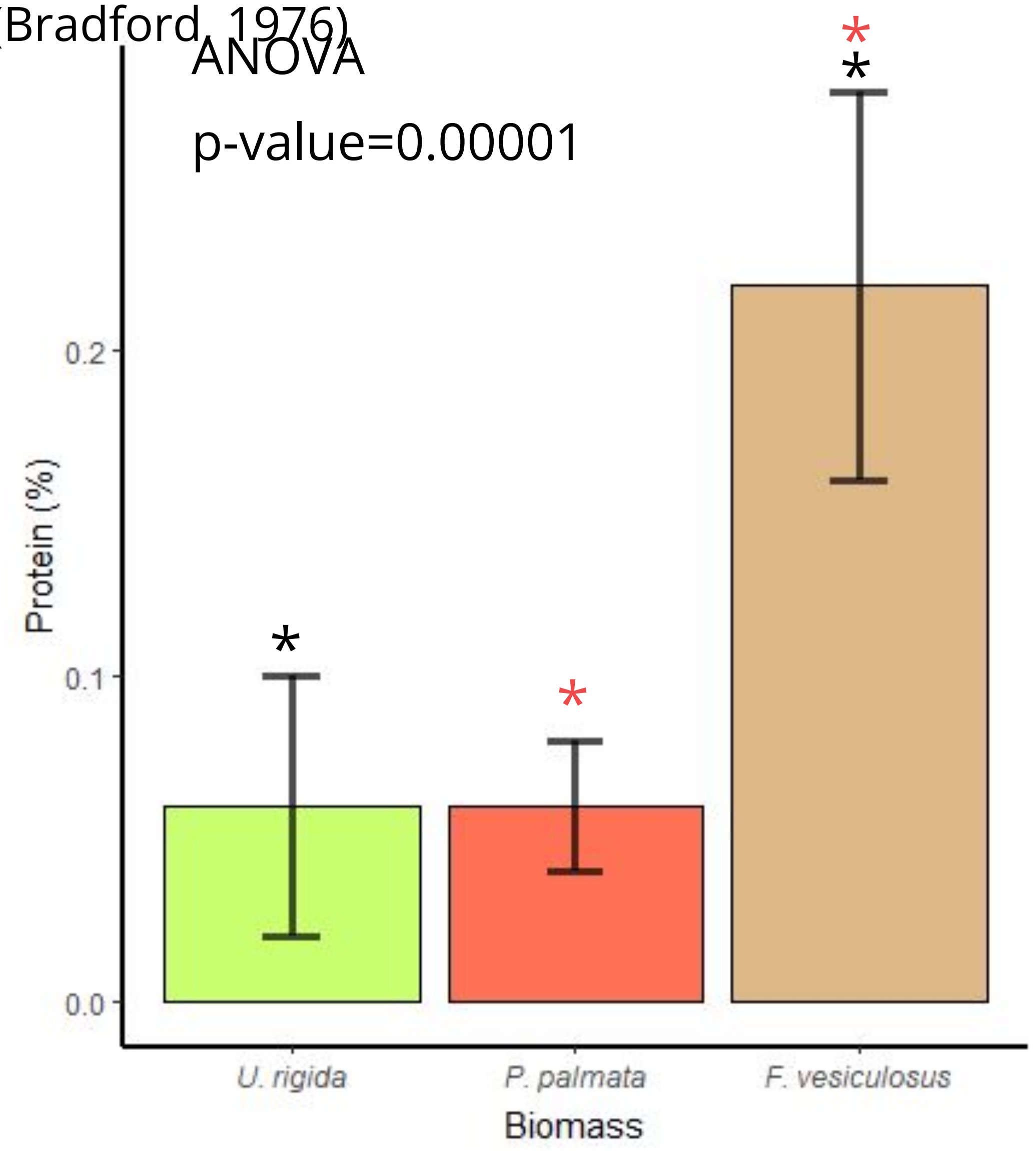
7. Snack acceptance


8. Market survey

# NUTRICIONAL PROFILE - TOTAL PROTEINS

Extracted with 80% ethanol, 80ºC ultrasound

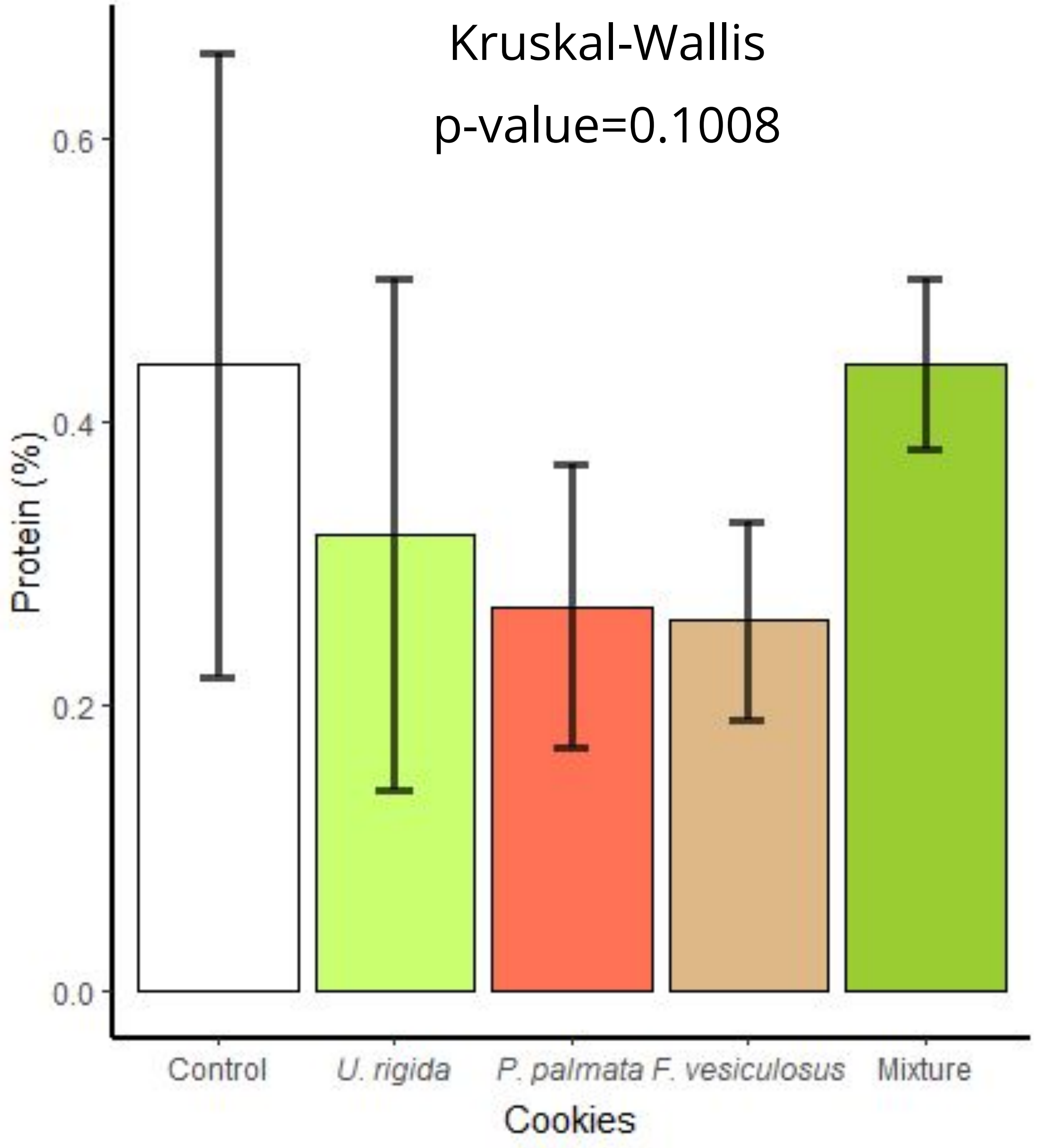
Bradford method (Bradford, 1976)




**Tukey p-value**

U. rigida vs F. vesiculosus **0.0003**

P. palmata vs F. vesiculosus **0.00001**



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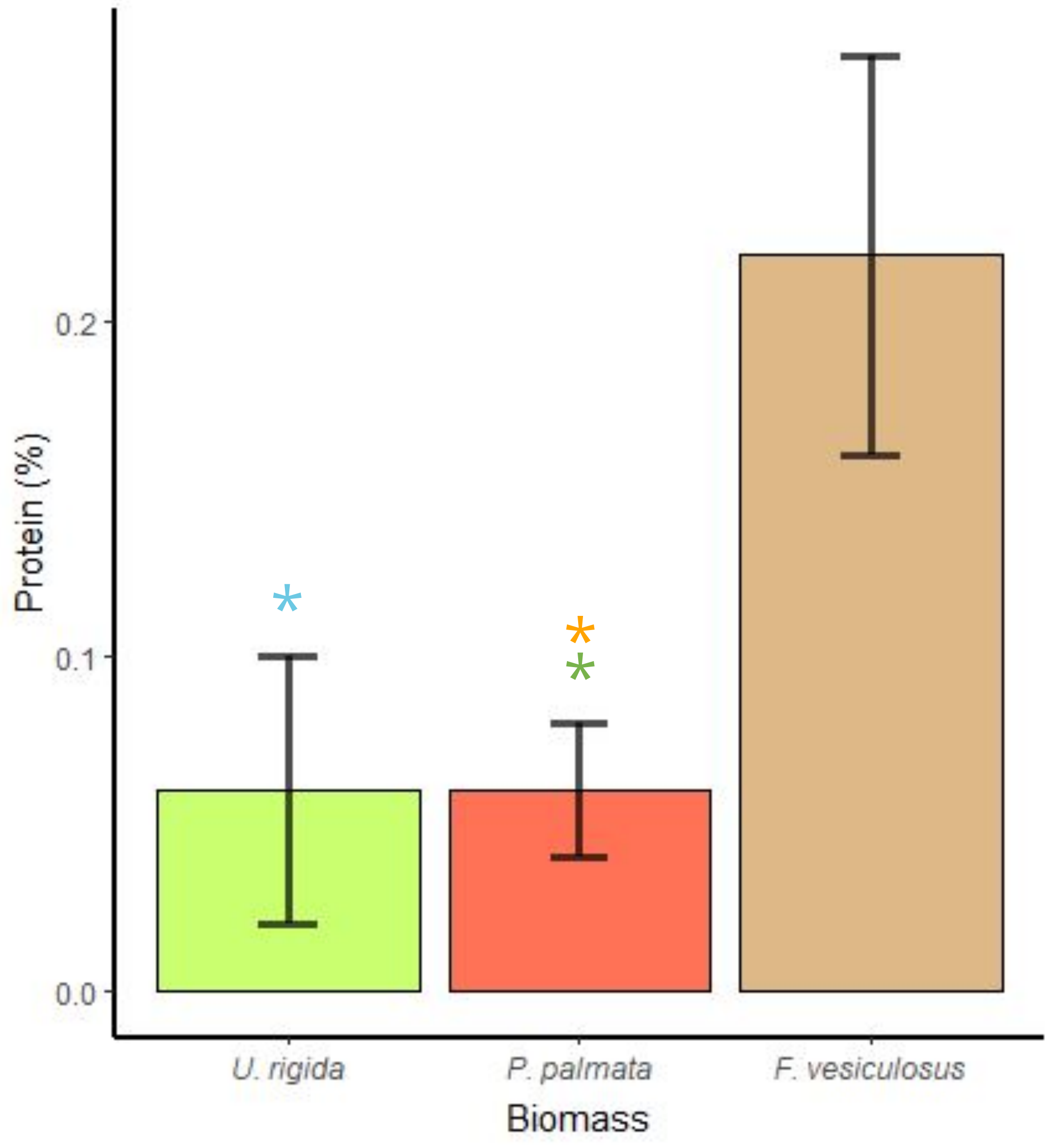
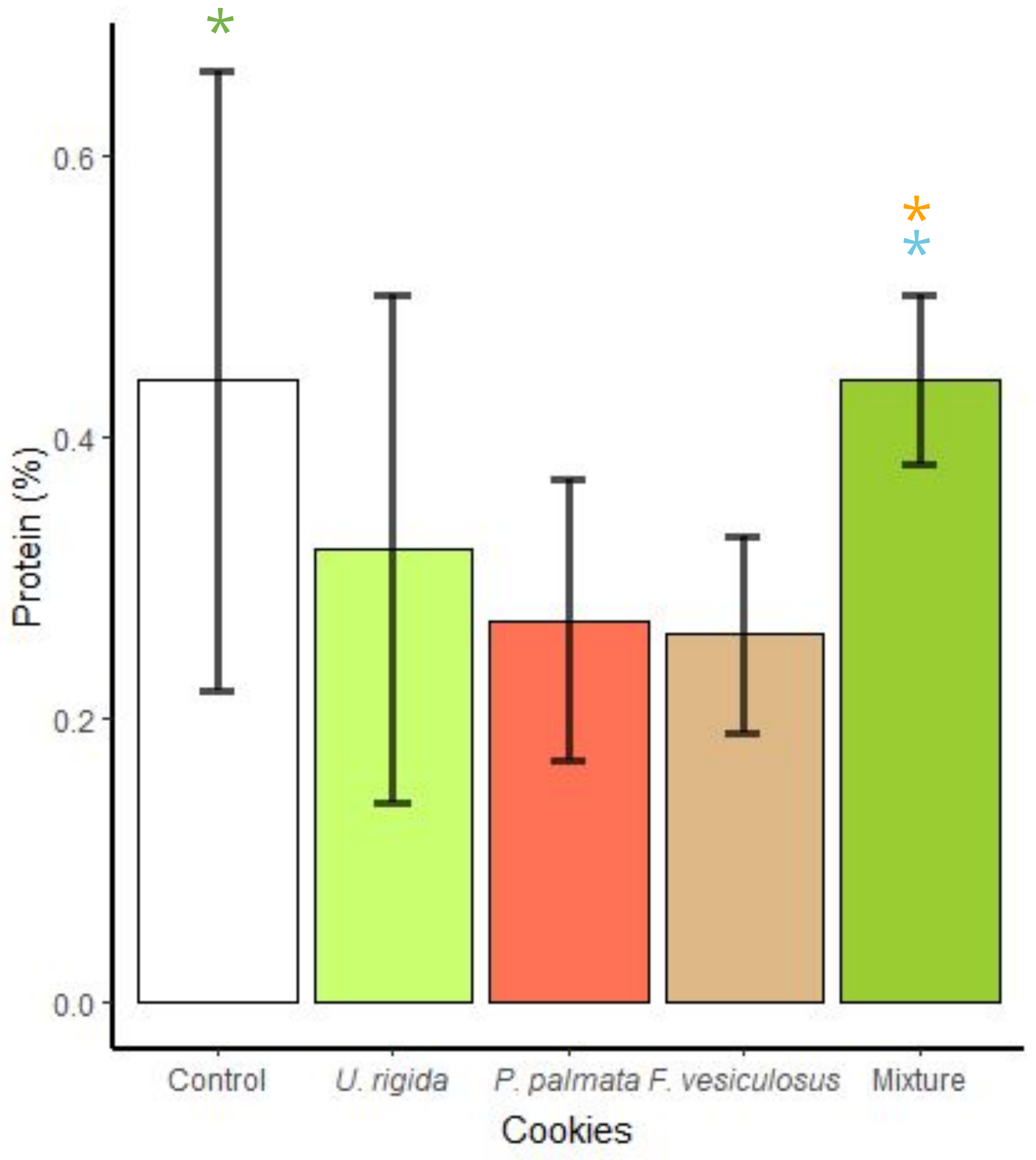



Kruskal-Wallis p-value=0.00006

| **Dunn (BONFERRONI) p-value** | |
|---|---|
| U. rigida vs Mixture | 0.002 |
| P. palmata vs Control | 0.003 |
| P. palmata vs Mixture | 0.0001 |



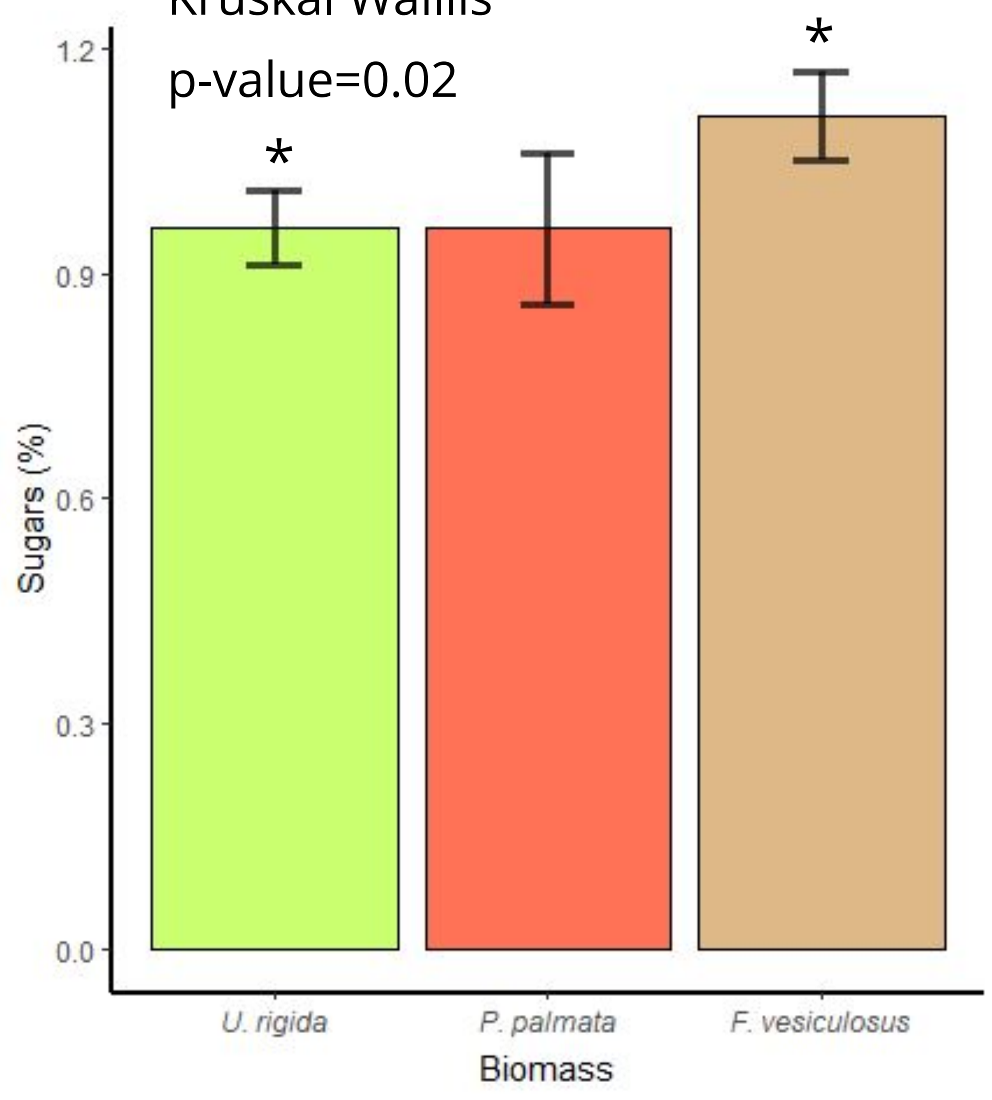
# NUTRICIONAL PROFILE - SUGARS

Extracted with 80% ethanol, 80ºC ultrasound

Extract mixed with  dH2O, 9% phenol and 96% sulphuric acid.

Kruskal Walllis

p-value=0.02




**Dunn (BONFERRONI) p-value**

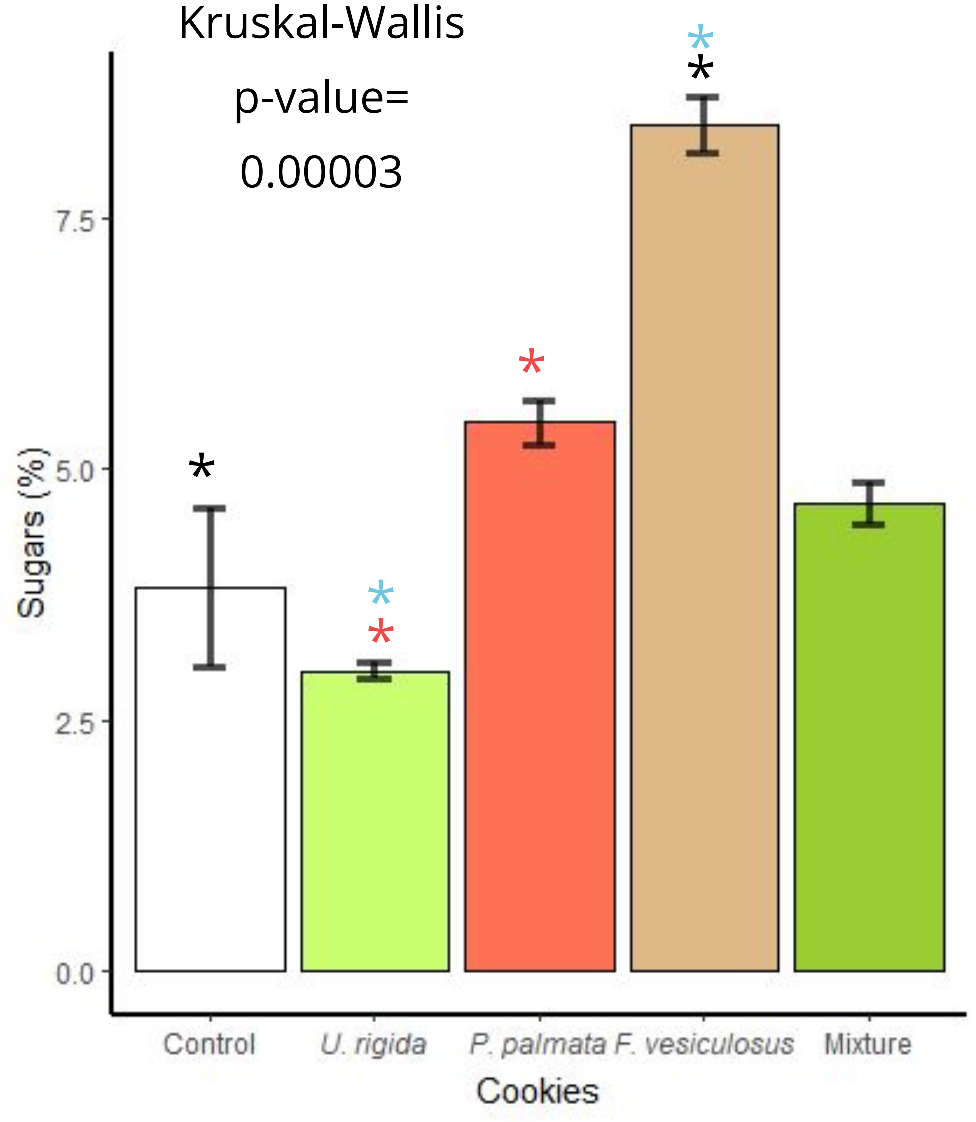
U. rigida vs F. vesiculosus

**0.02**

Kruskal-Wallis

p-value=

0.00003




**Dunn (BONFERRONI) p-value**

Control vs F. vesiculosus cookies

**0.0024**

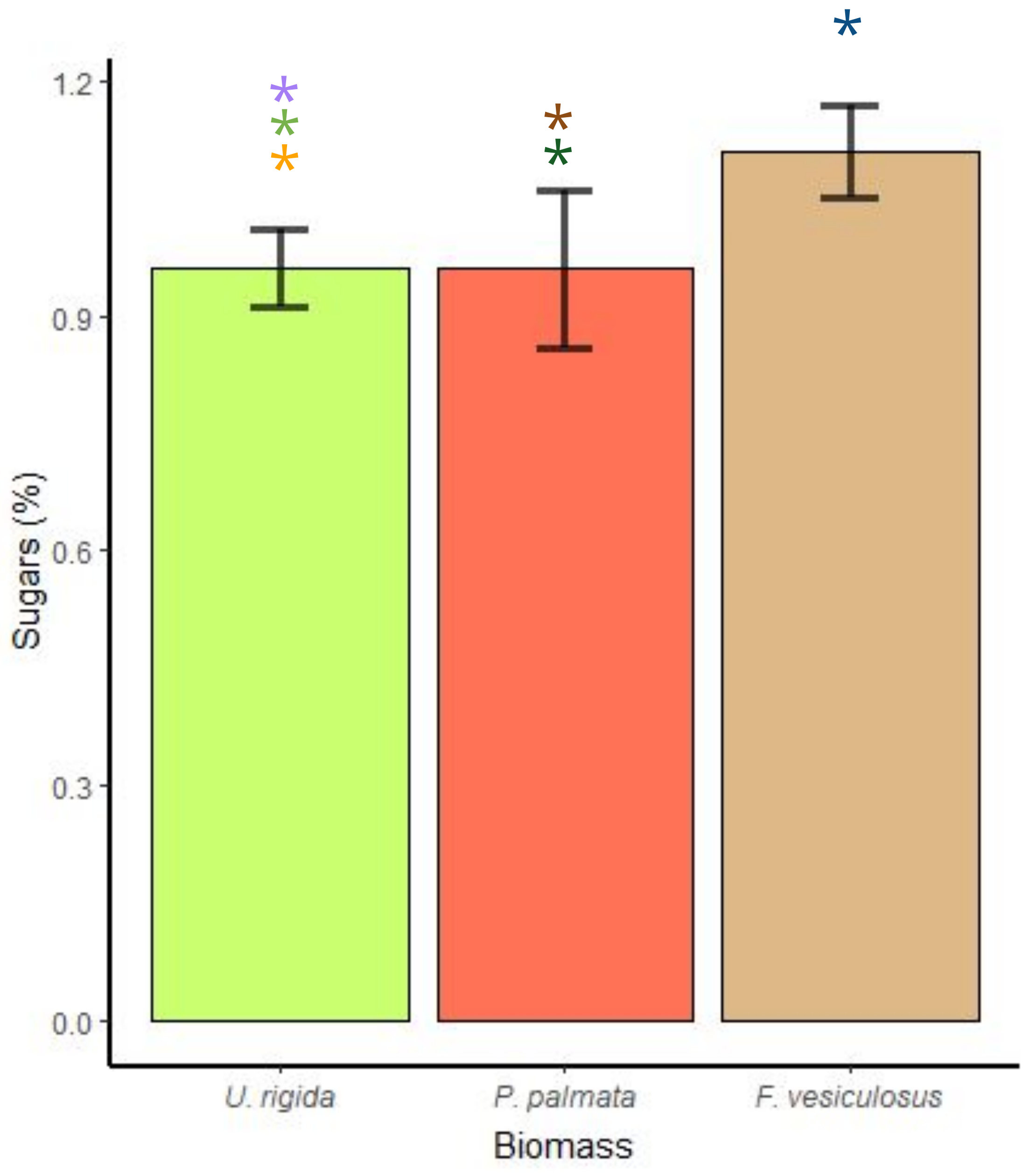
U. rigida cookies vs P. palmata cookies **0.004**

U. rigida cookies vs F. vesiculosus cookies **~0**

# NUTRICIONAL PROFILE - SUGARS

Extracted with 80% ethanol, 80ºC ultrasound

Extract mixed with dH2O, 9% phenol and 96% sulphuric acid.





Kruskal-Wallis p-value=~0

**Dunn (BONFERRONI) p-value**

U. rigida vs P. palmata cookies 0.0007

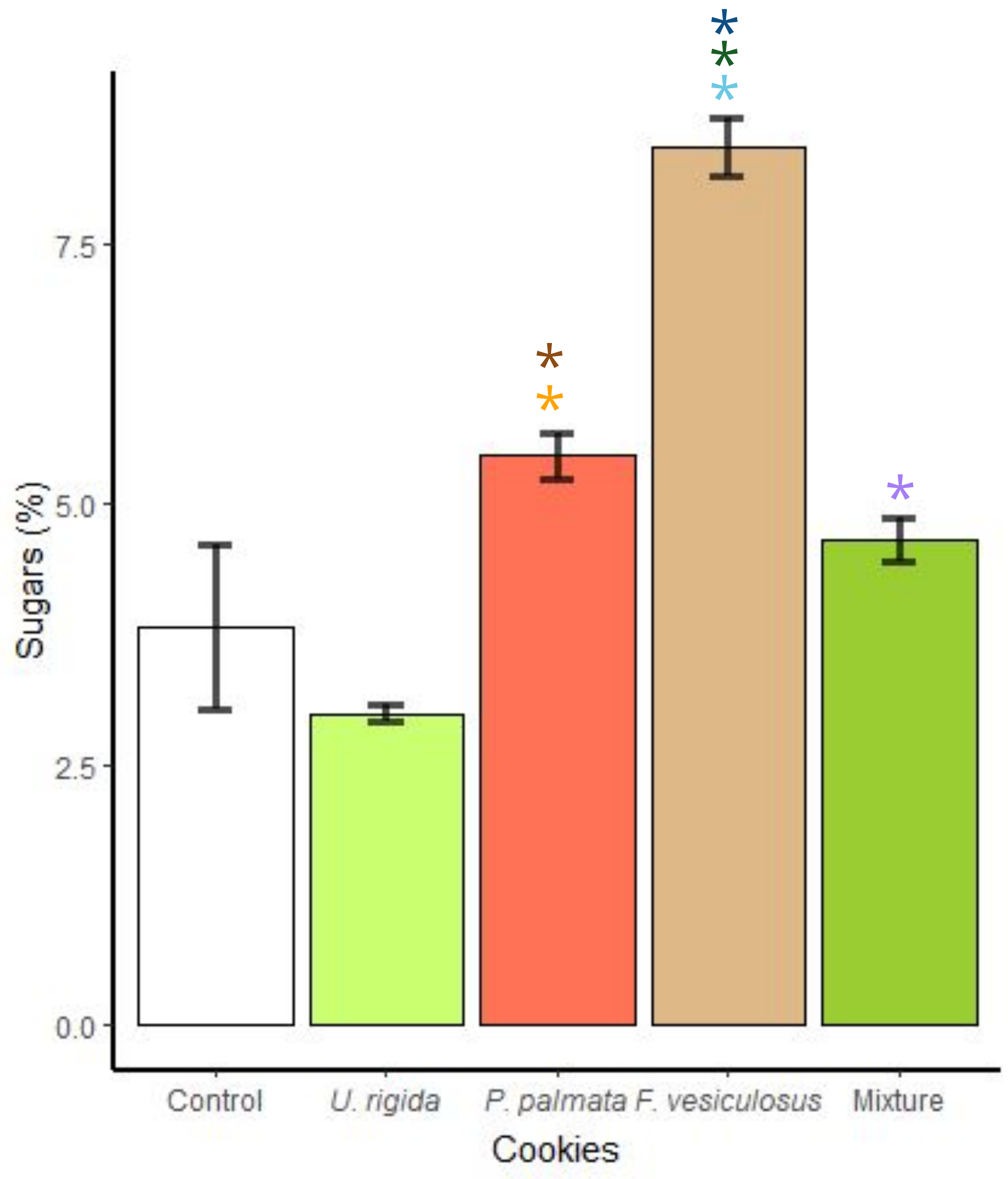
U. rigida vs F. vesiculosus cookies ~0

U. rigida vs Mixture 0.2

P. palmata vs P. palmata cookies 0.001

P. palmata vs F. vesiculosus cookies ~0

F. vesiculosus vs F. vesiculosus cookies 0.002

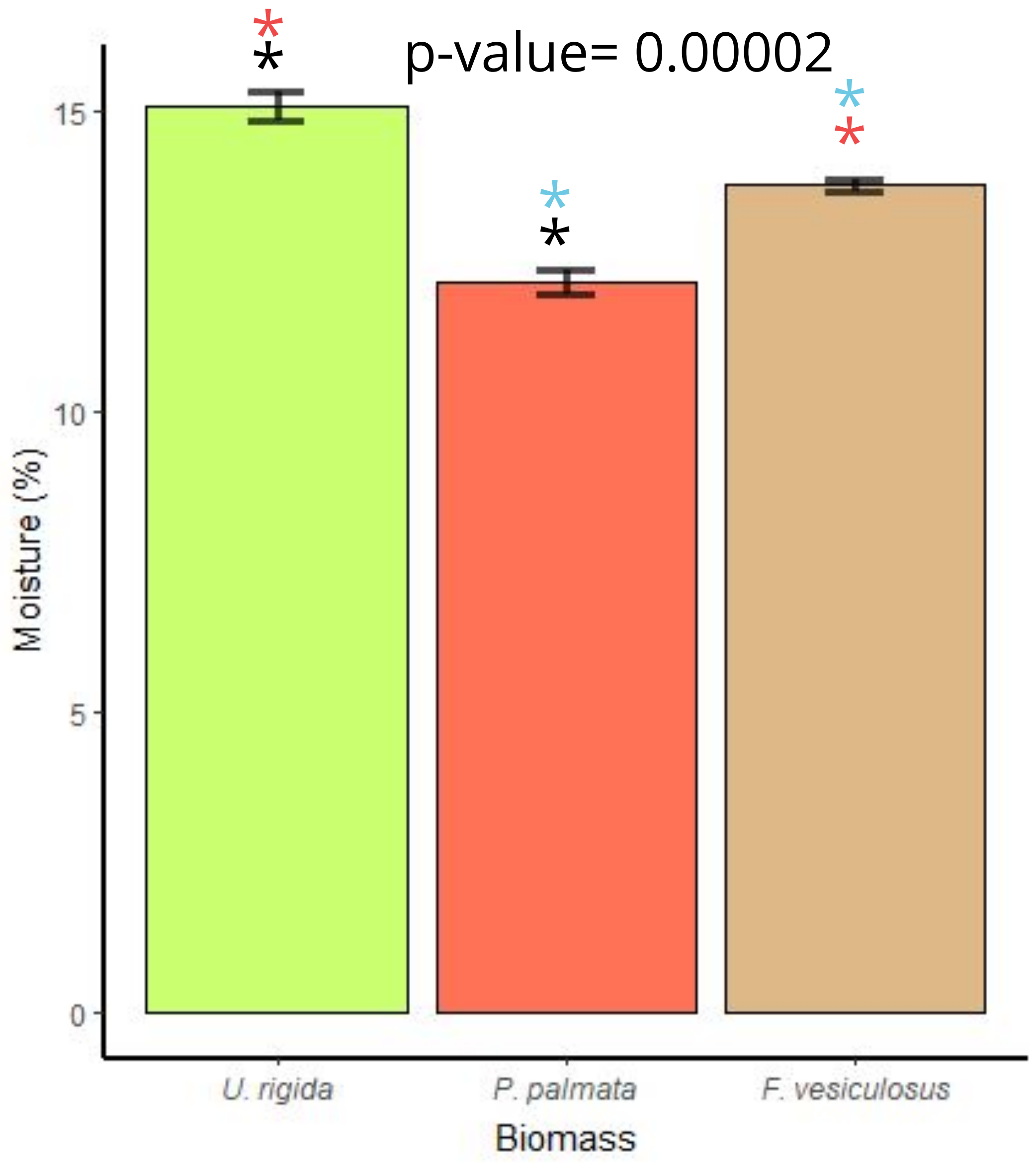


# NUTRICIONAL PROFILE - MOISTURE

105 ºC for 24 h

ANOVA

p-value= 0.00002




**Tukey p-value**

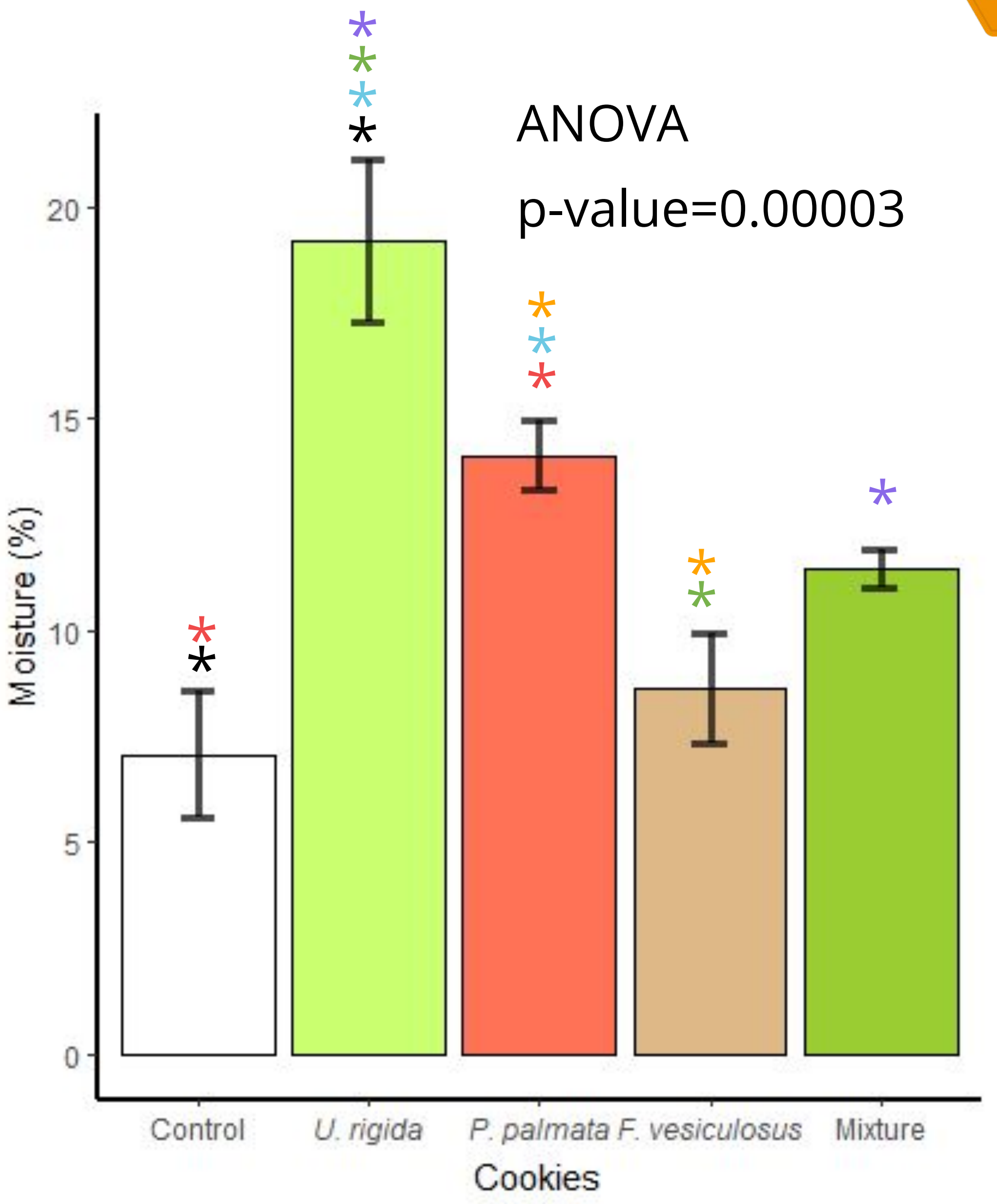
U. rigida vs P. palmata **0.00001**

U. rigida vs F. vesiculosus **0.001**

P. palmata vs F. vesiculosus **0.0004**

ANOVA

p-value=0.00003




**Tukey p-value**

Control vs U. rigida cookies **0.00003**

Control vs P. palmata cookies **0.002**

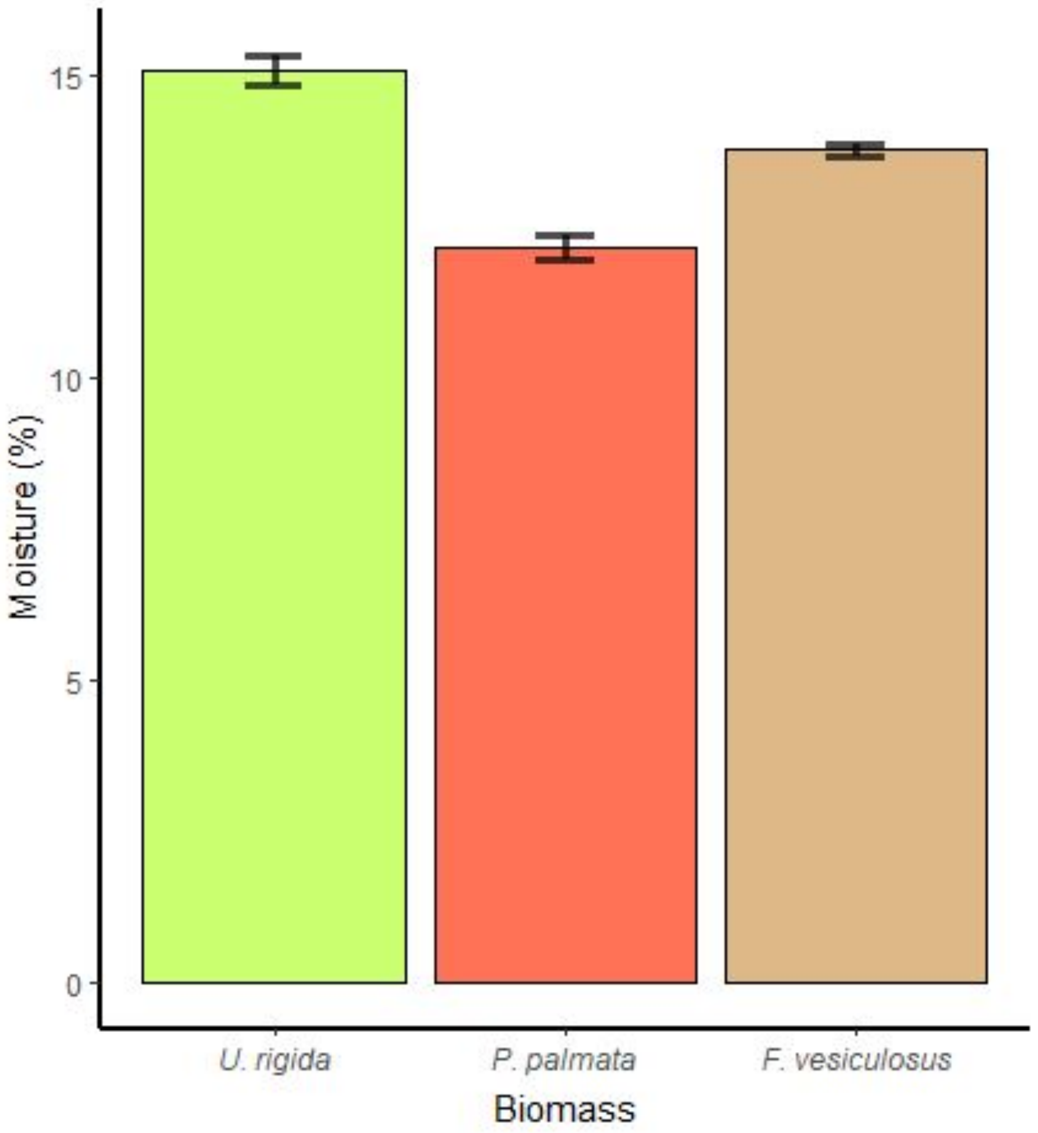
U. rigida cookies vs P. palmata cookies **0.02**

U. rigida cookies vs F. vesiculosus cookies **0.00008**

U. rigida cookies vs Mixture **0.001**

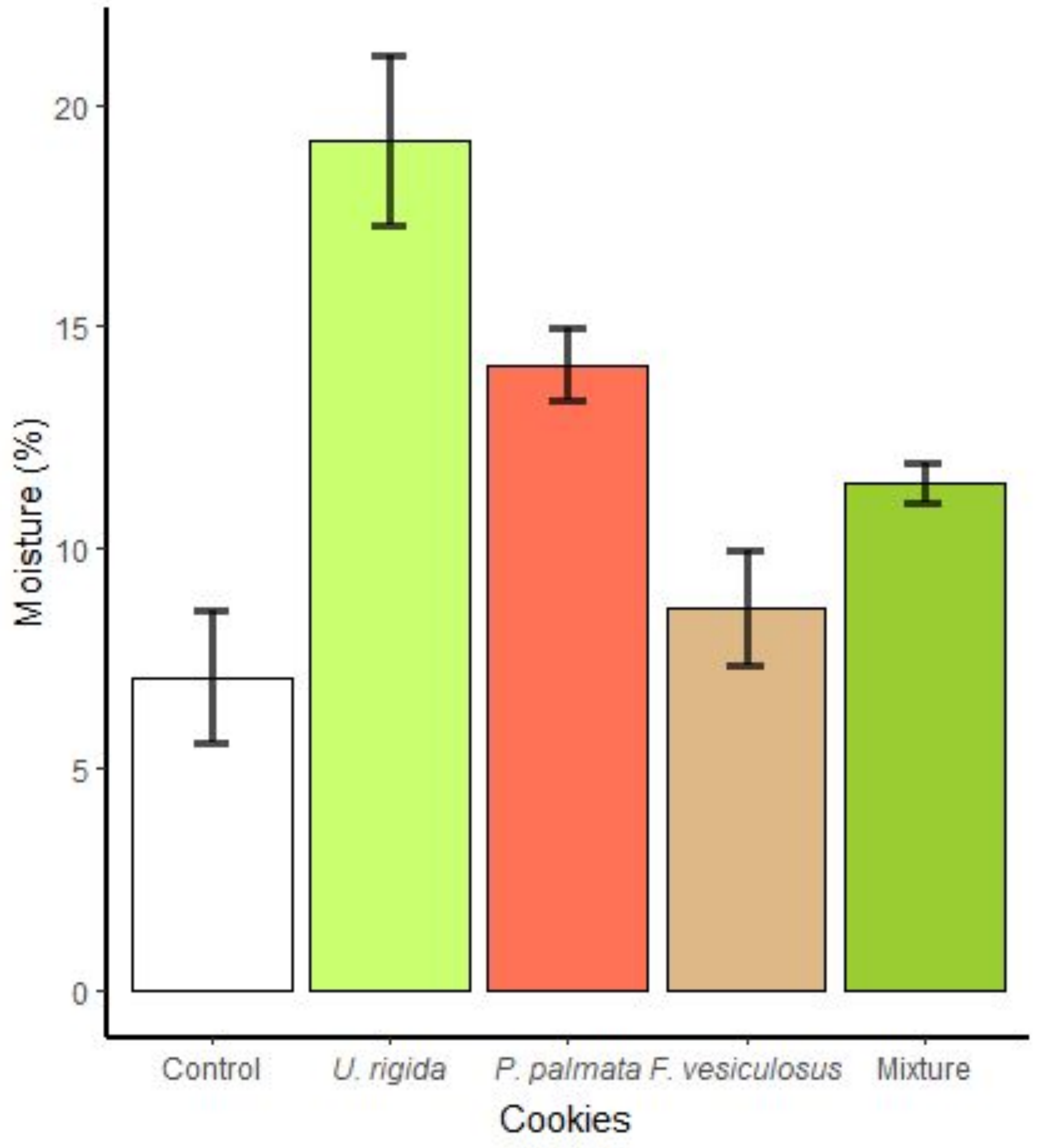
# NUTRICIONAL PROFILE - MOISTURE

105 ºC for 24 h



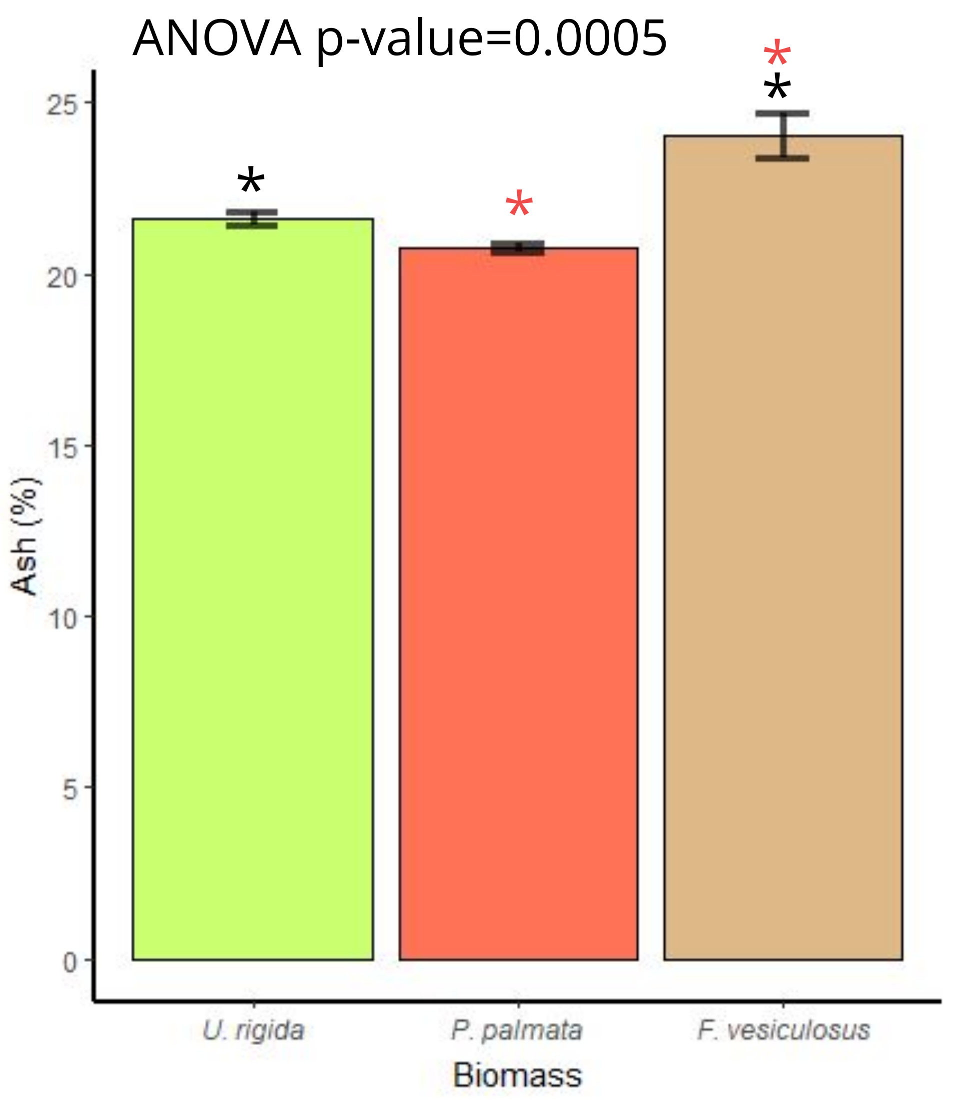

Kruskal Wallis p-value=~0

**Dunn (BONFERRONI) p-value>0.05**



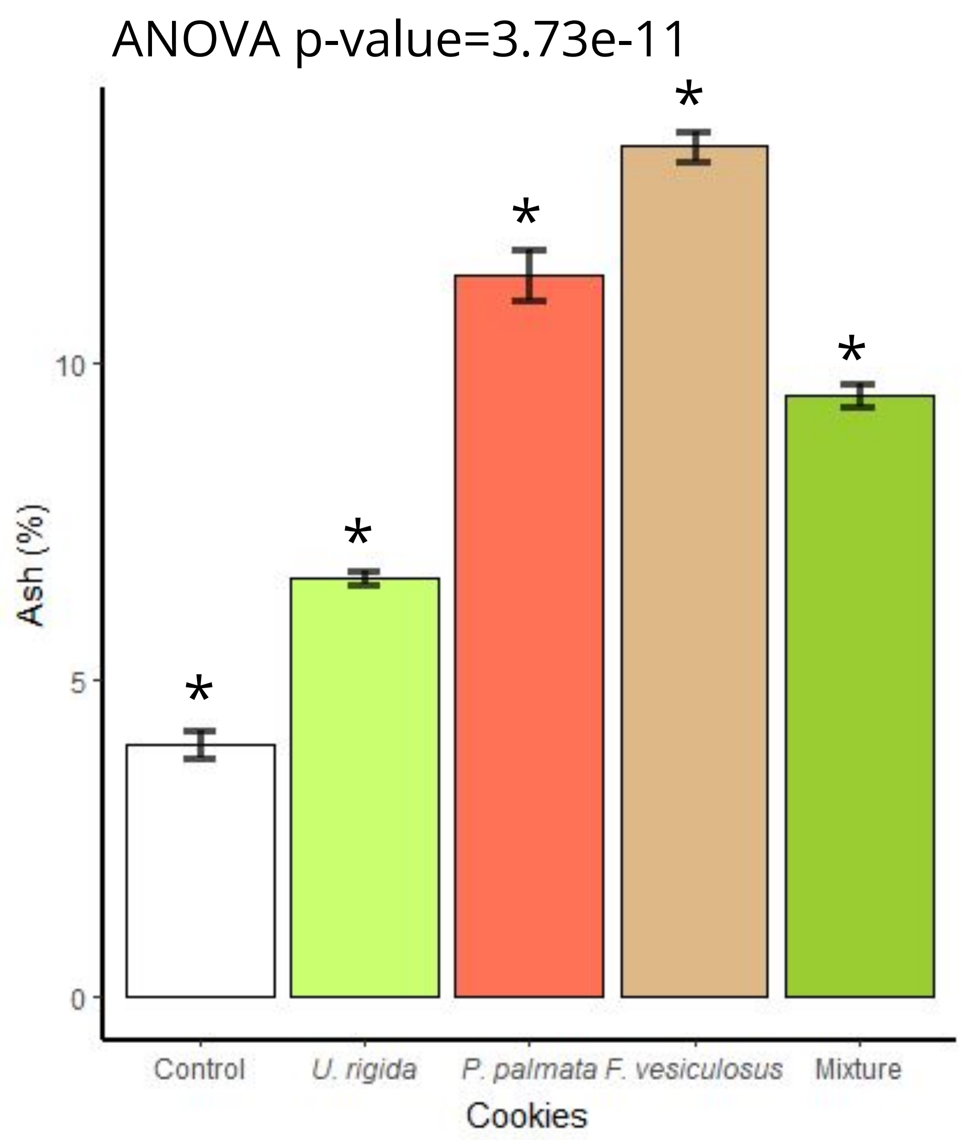
# NUTRICIONAL PROFILE - ASH

515 ºC for 4 h




**Tukey p-value**

| | |
|---|---|
| U. rigida vs F. vesiculosus | **0.002** |
| P. palmata vs F. vesiculosus | **0.0004** |

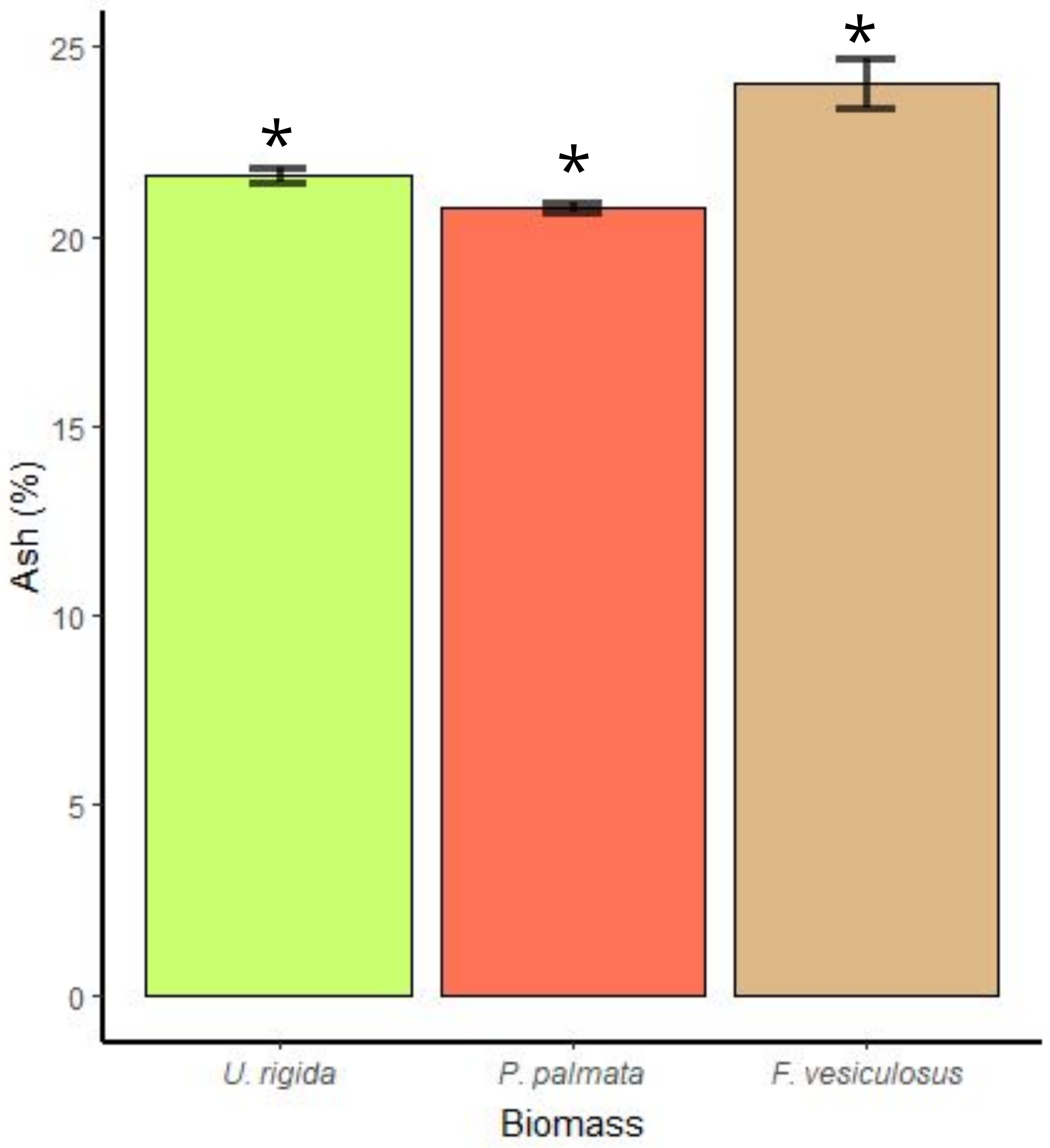



**Tukey p-value**

| | |
|---|---|
| Control vs U. rigida cookies | **0.00001** |
| Control vs P. palmata cookies | **~0** |
| Control vs F. vesiculosus cookies | **~0** |
| Control vs Mixture | **~0** |
| U. rigida cookies vs P. palmata cookies | **~0** |
| U. rigida cookies vs F. vesiculosus cookies | **~0** |
| U. rigida cookies vs Mixture | **0.000004** |
| P. palmata cookies vs F. vesiculosus cookies | **0.00009** |
| P. palmata cookies vs Mixture | **0.0002** |

# NUTRICIONAL PROFILE - ASH

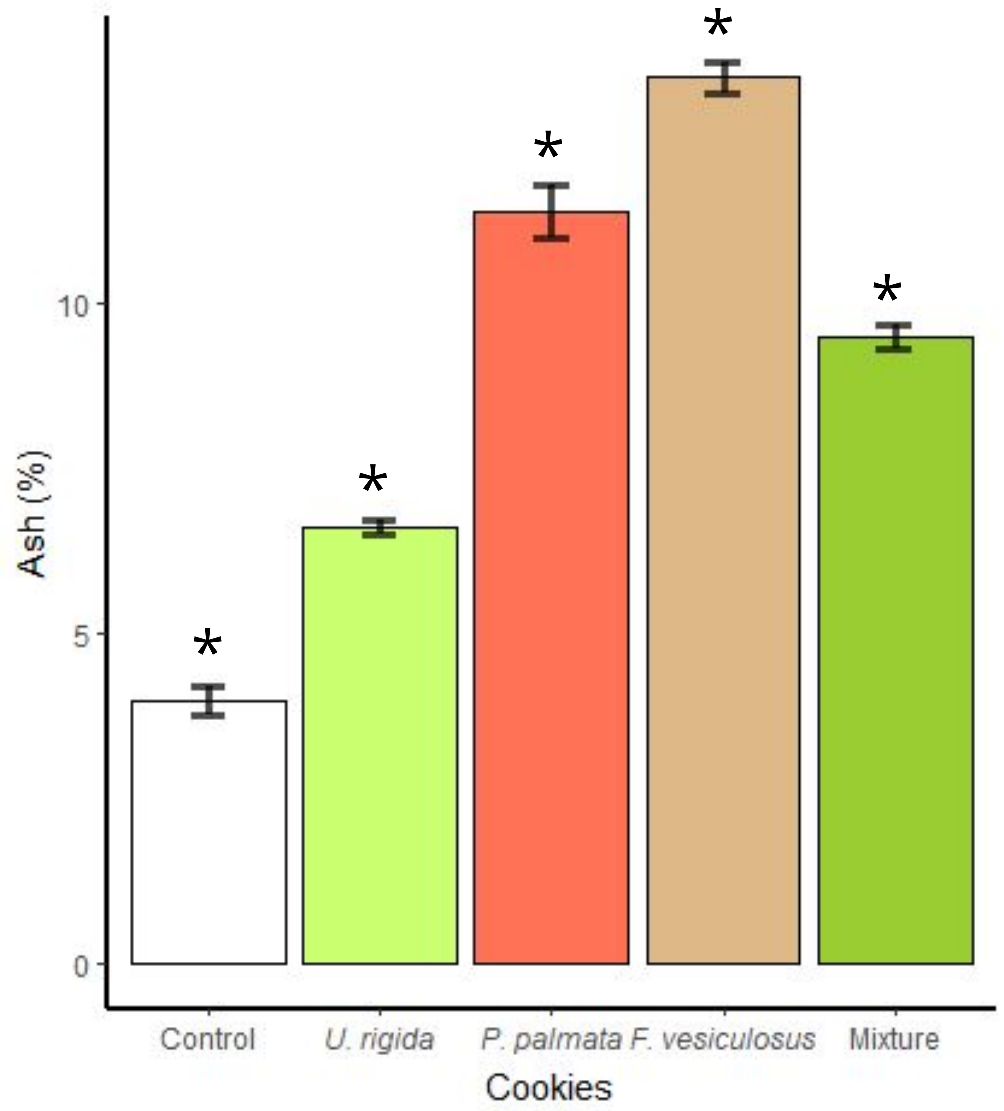
515 ºC for 4 h




ANOVA p-value= <2e-16

**Tukey p-value**

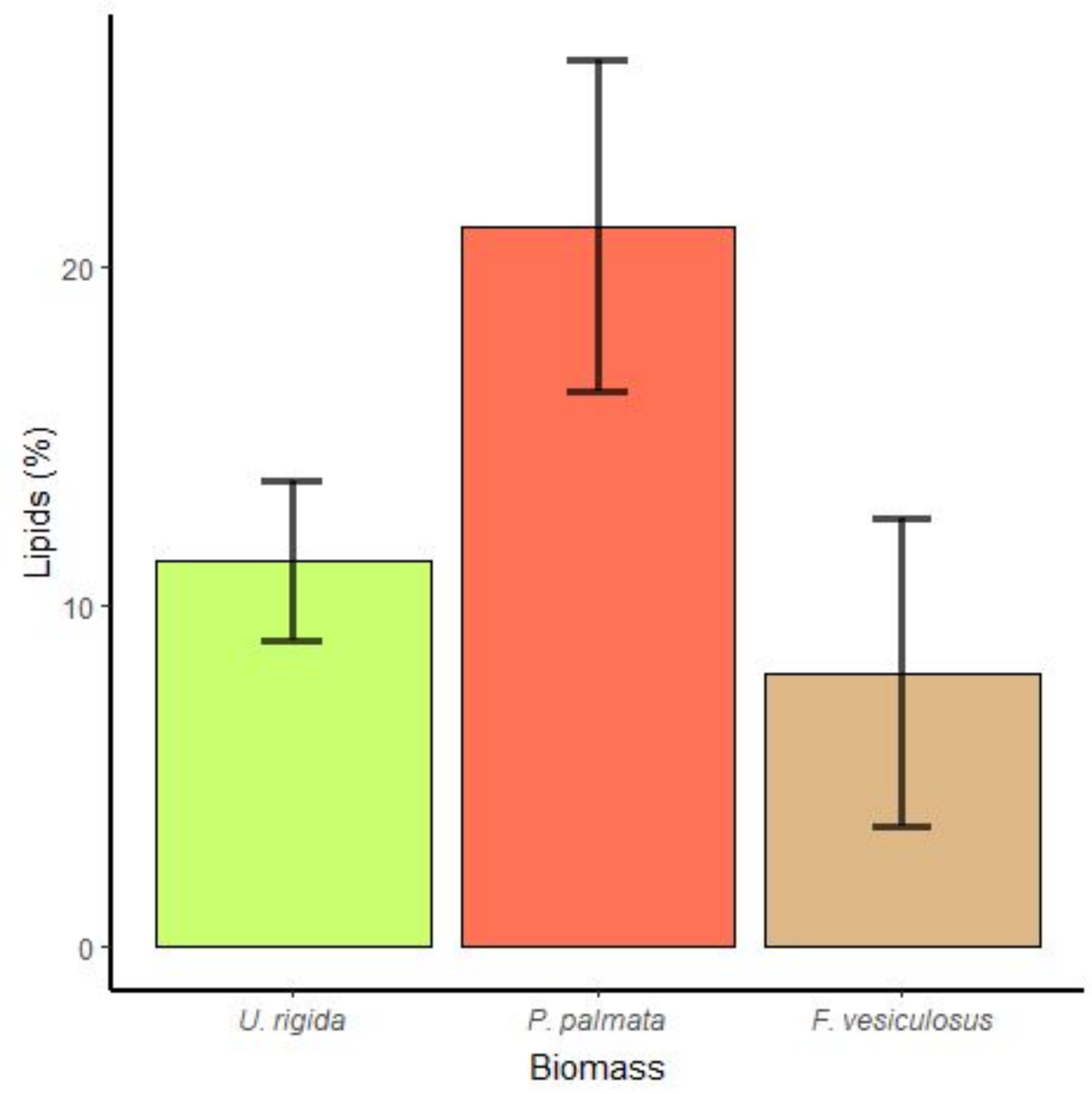
**All values <e-7**

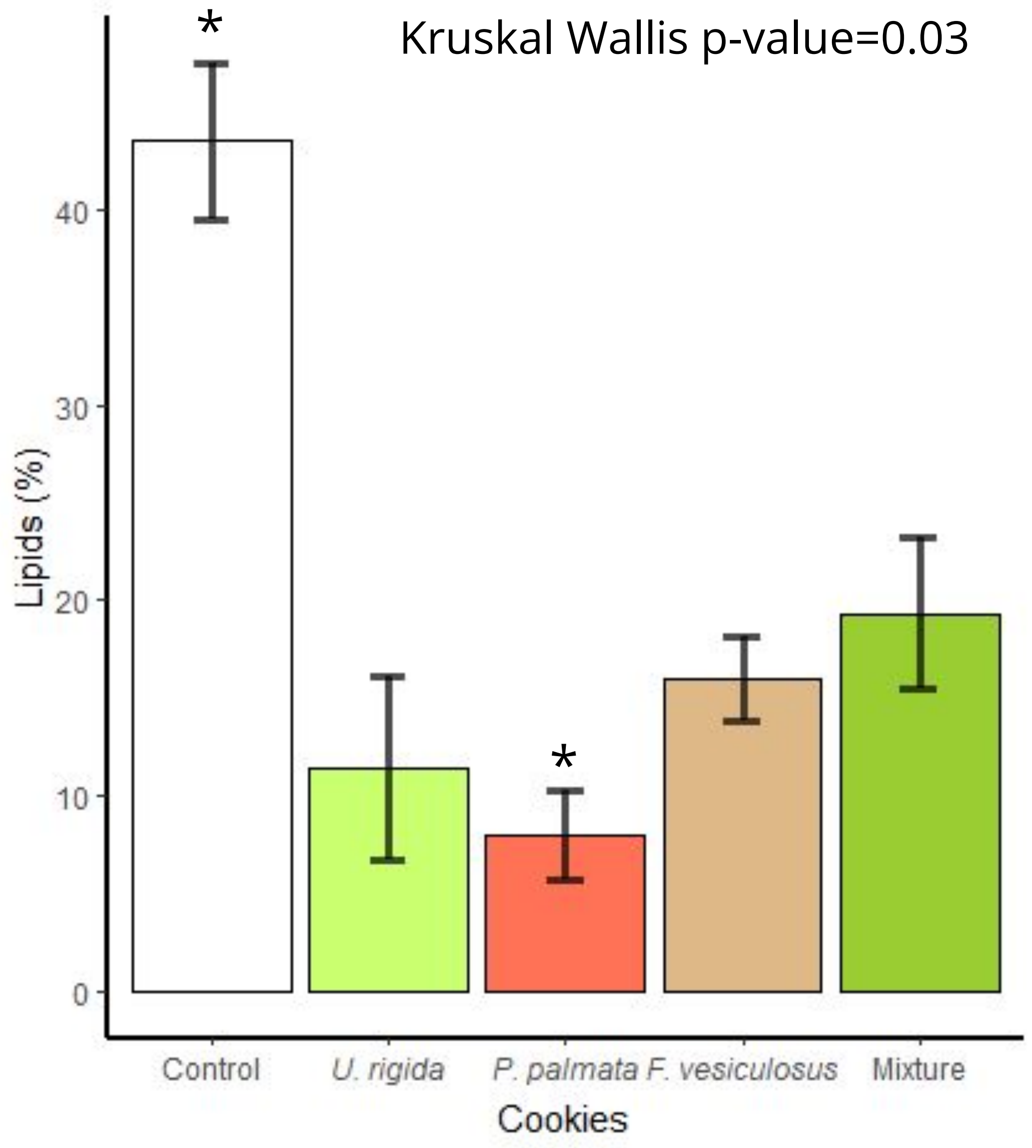


# NUTRICIONAL PROFILE - LIPIDS

Bligh & Dyer modified protocol (1959)

Kruskal Wallis p-value=0.1456

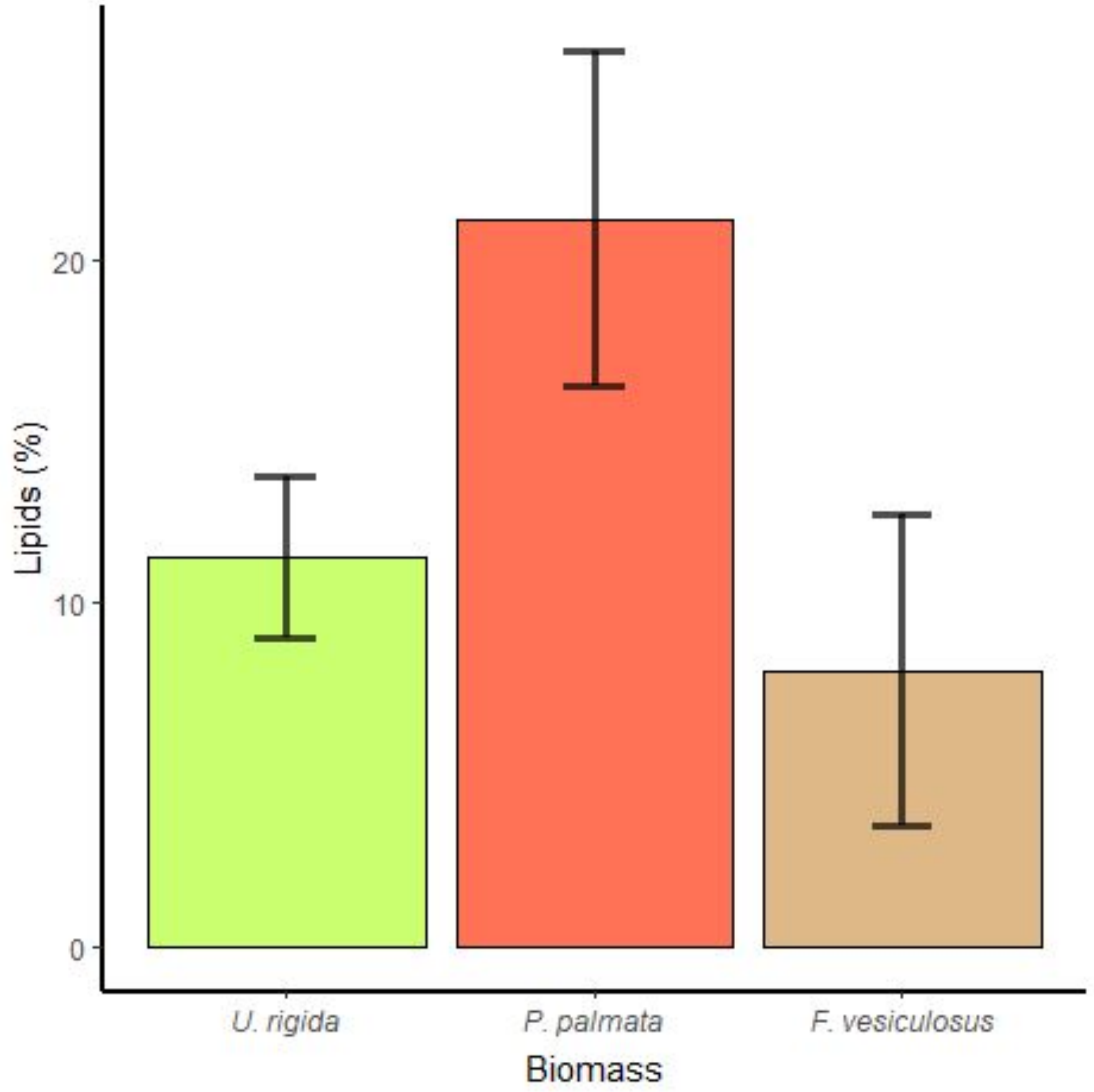



| **Dunn (BONFERRONI) p-value** | |
|---|---|
| Control vs P. palmata cookies | **0.009** |

# NUTRICIONAL PROFILE - LIPIDS

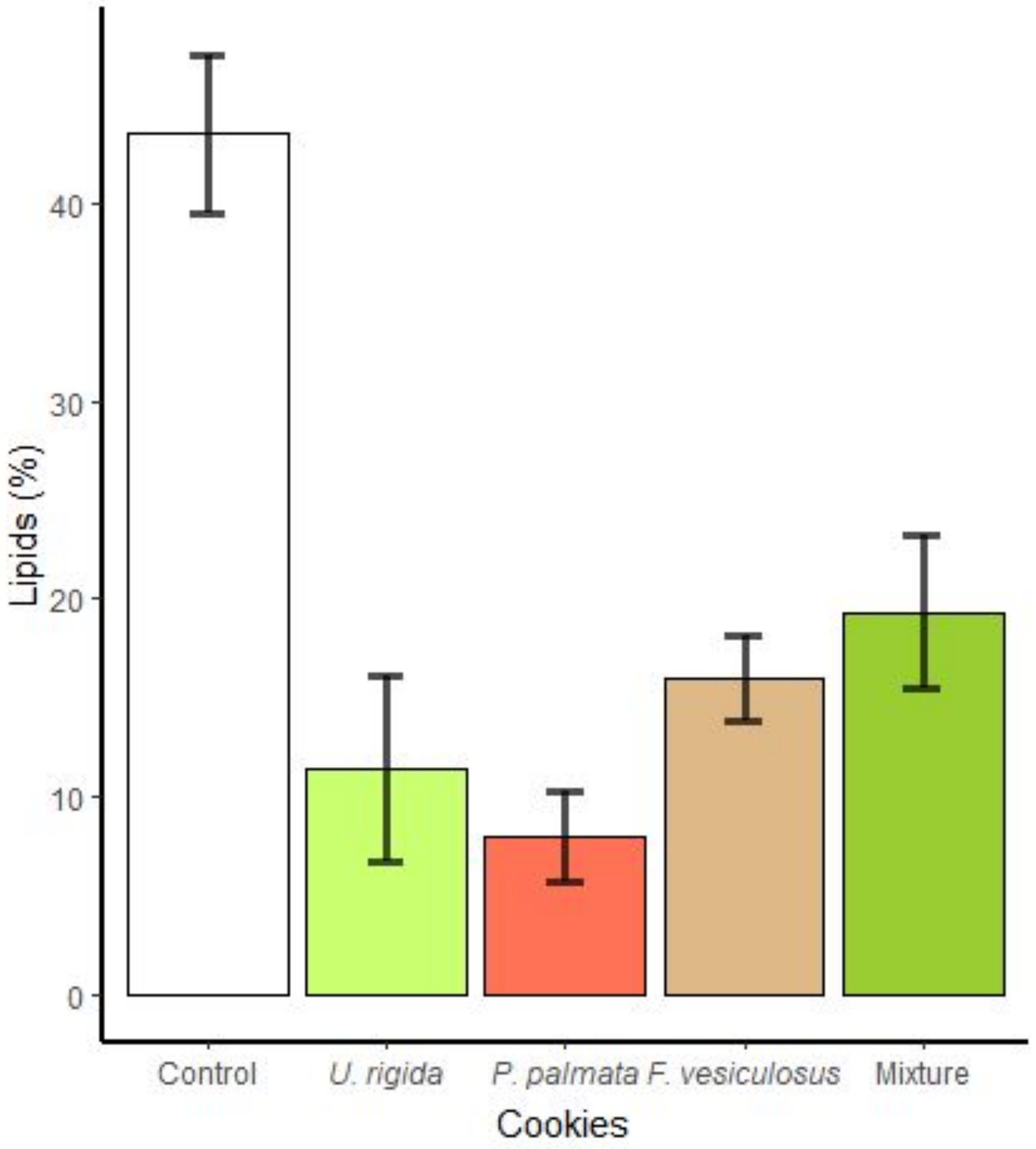
Bligh & Dyer modified protocol (1959)



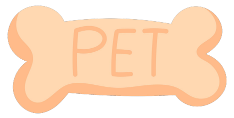
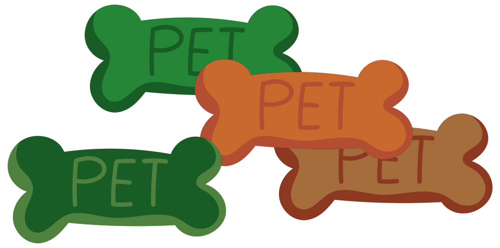


Kruskal Wallis p-value=0.02

**Dunn (BONFERRONI) p-value >0.05**

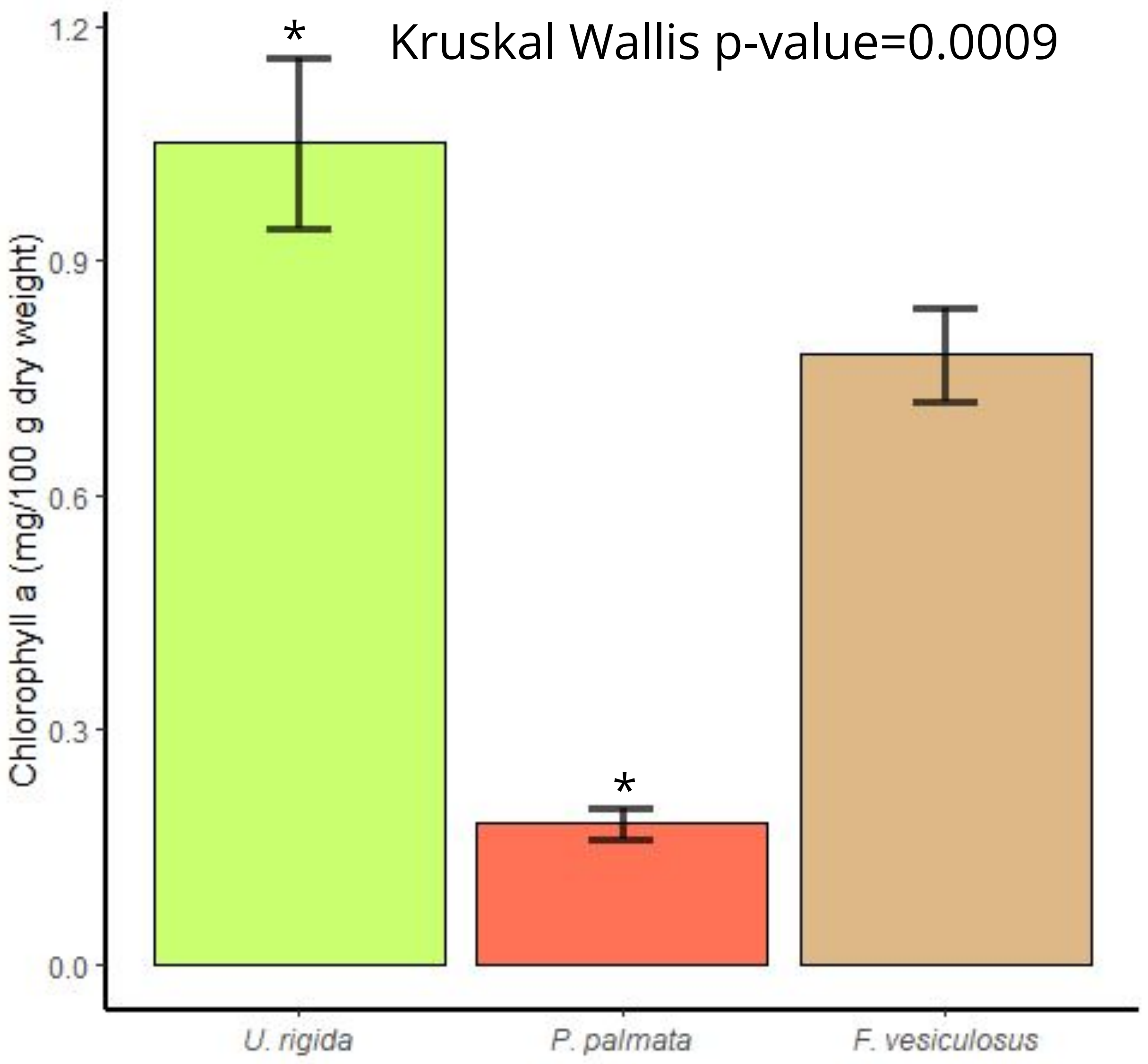


# NUTRICIONAL PROFILE - MINERALS

| | Macrominerals (mg/g) | Trace elements (µg/g) |
|---|---|---|
| Biomass  | K Mg Ca P Fe | Zn Mn Pb Cu Cd |
| Cookie Control  | P K Mg Ca Fe | Zn Mn Cu Pb Cd |
| Cookies  | K Mg P Ca Fe | Mn Zn Cu Pb Cd |

# NUTRICIONAL PROFILE - PIGMENTS

Biomass mixed with acetone-hexane (4:6), filtered



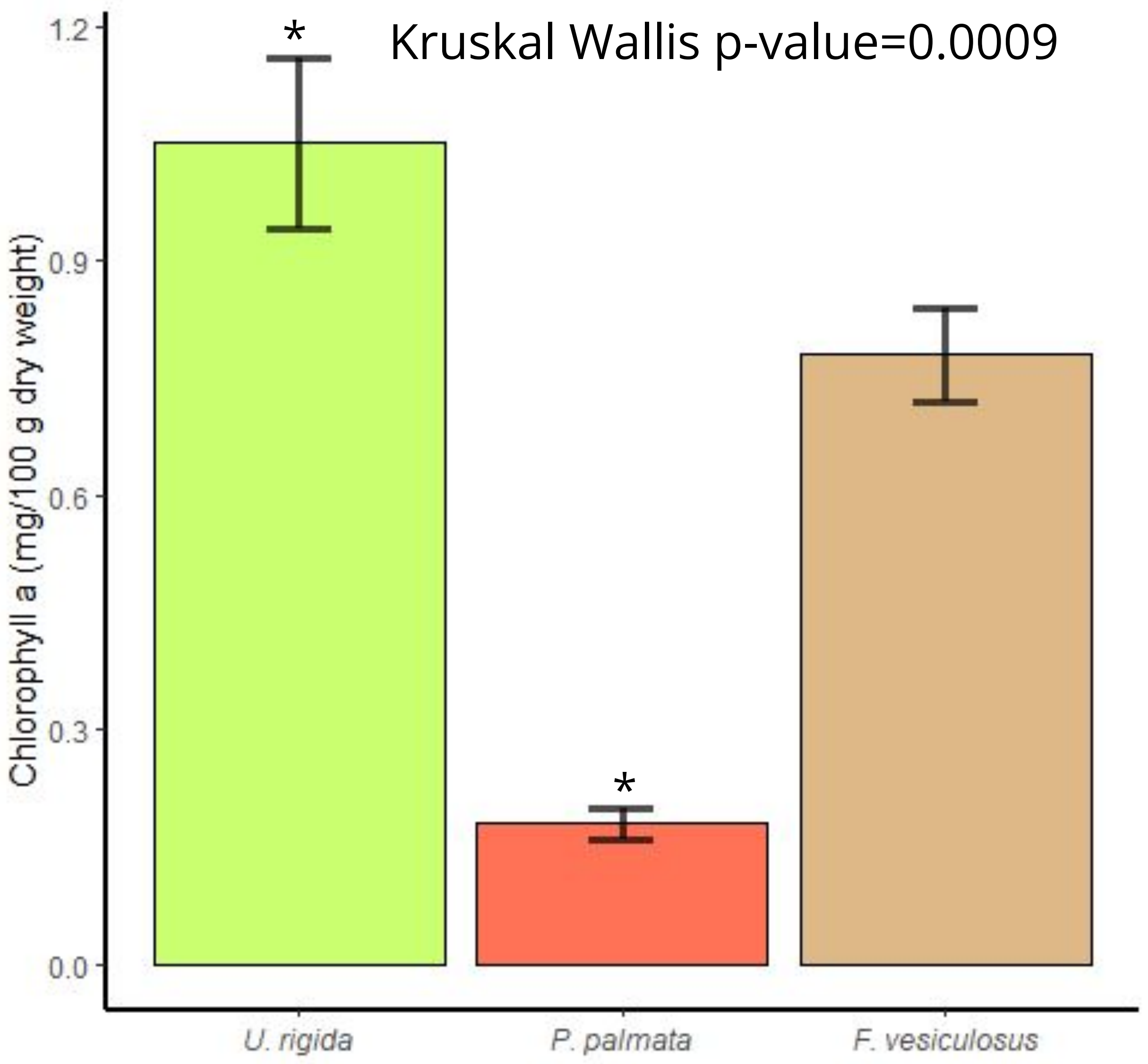

**Dunn (BONFERRONI) p-value**

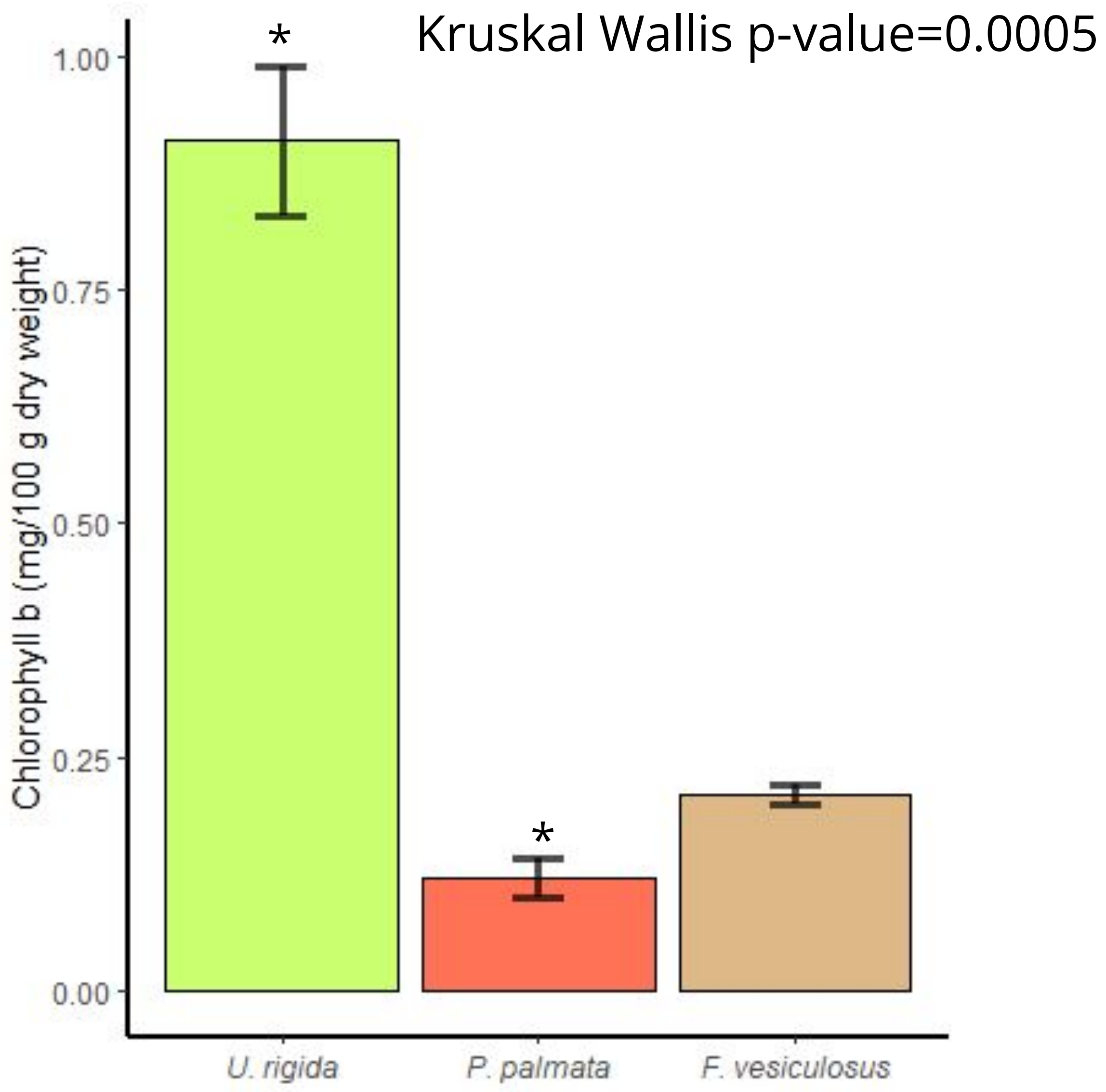
U. rigida vs P. palmata

**0.0003**

# NUTRICIONAL PROFILE - PIGMENTS

Biomass mixed with acetone-hexane (4:6), filtered




**Dunn (BONFERRONI) p-value**
U. rigida vs P. palmata
**0.0003**

**Dunn (BONFERRONI) p-value**
U. rigida vs P. palmata
**0.0001**

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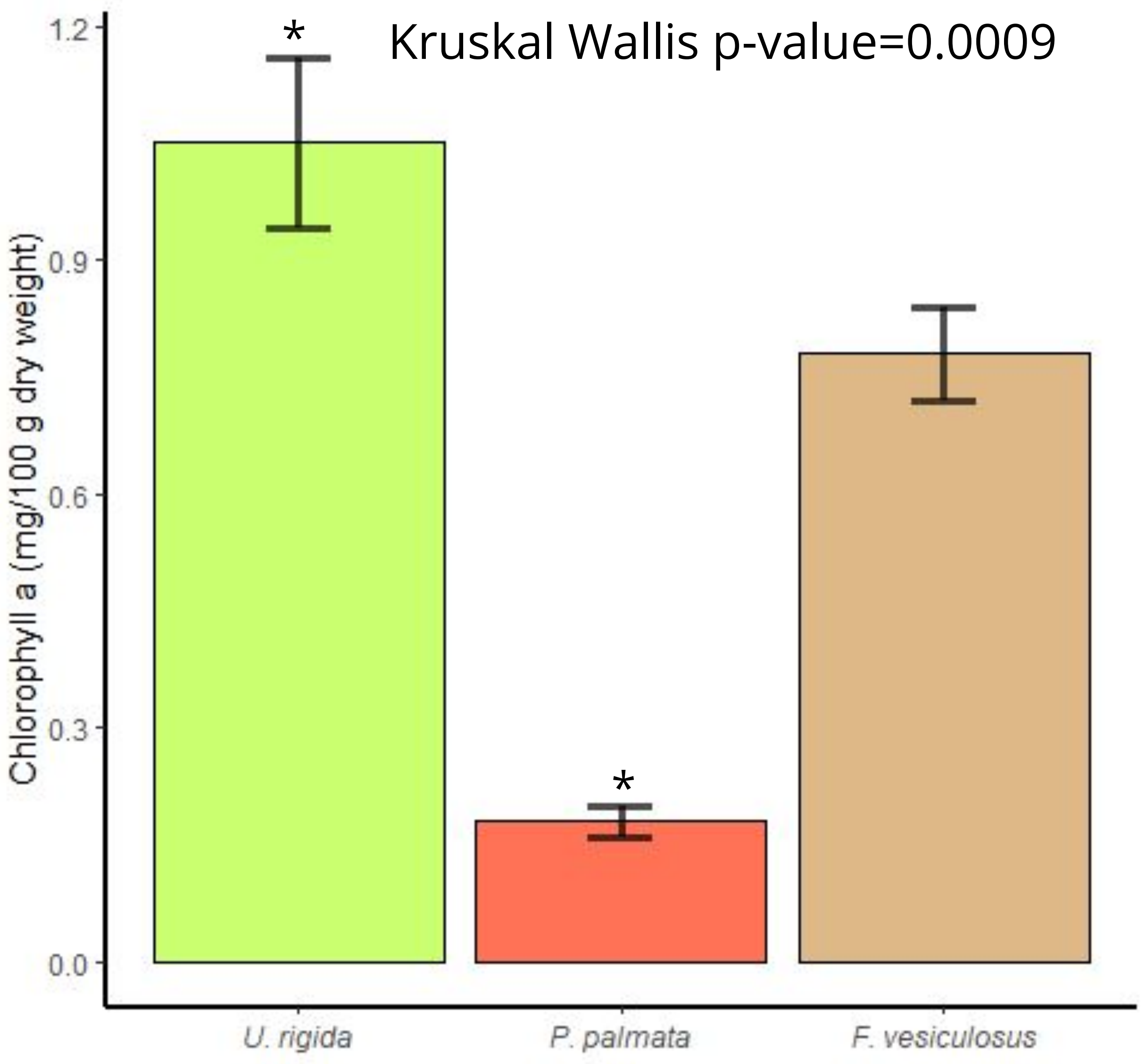
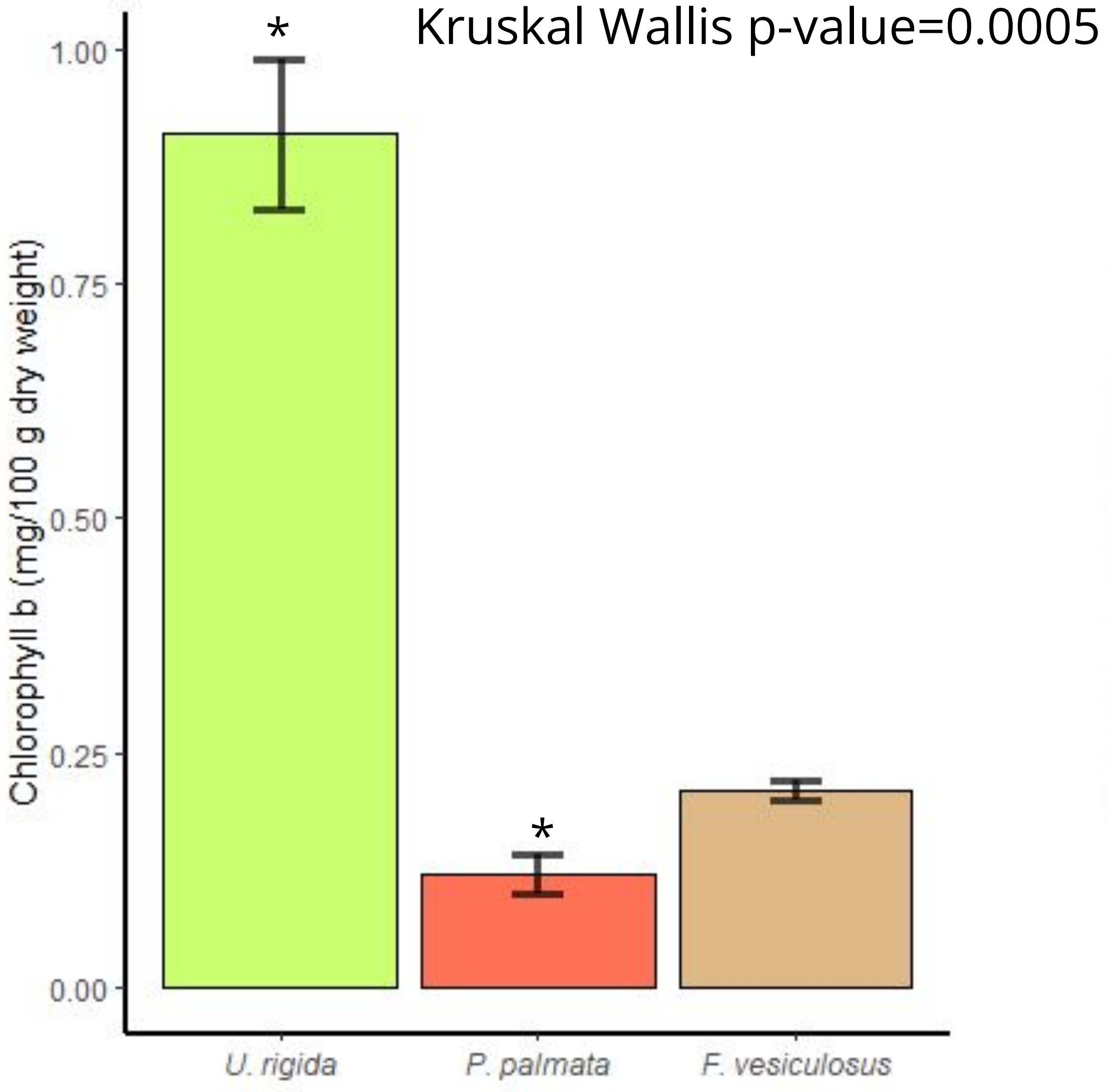
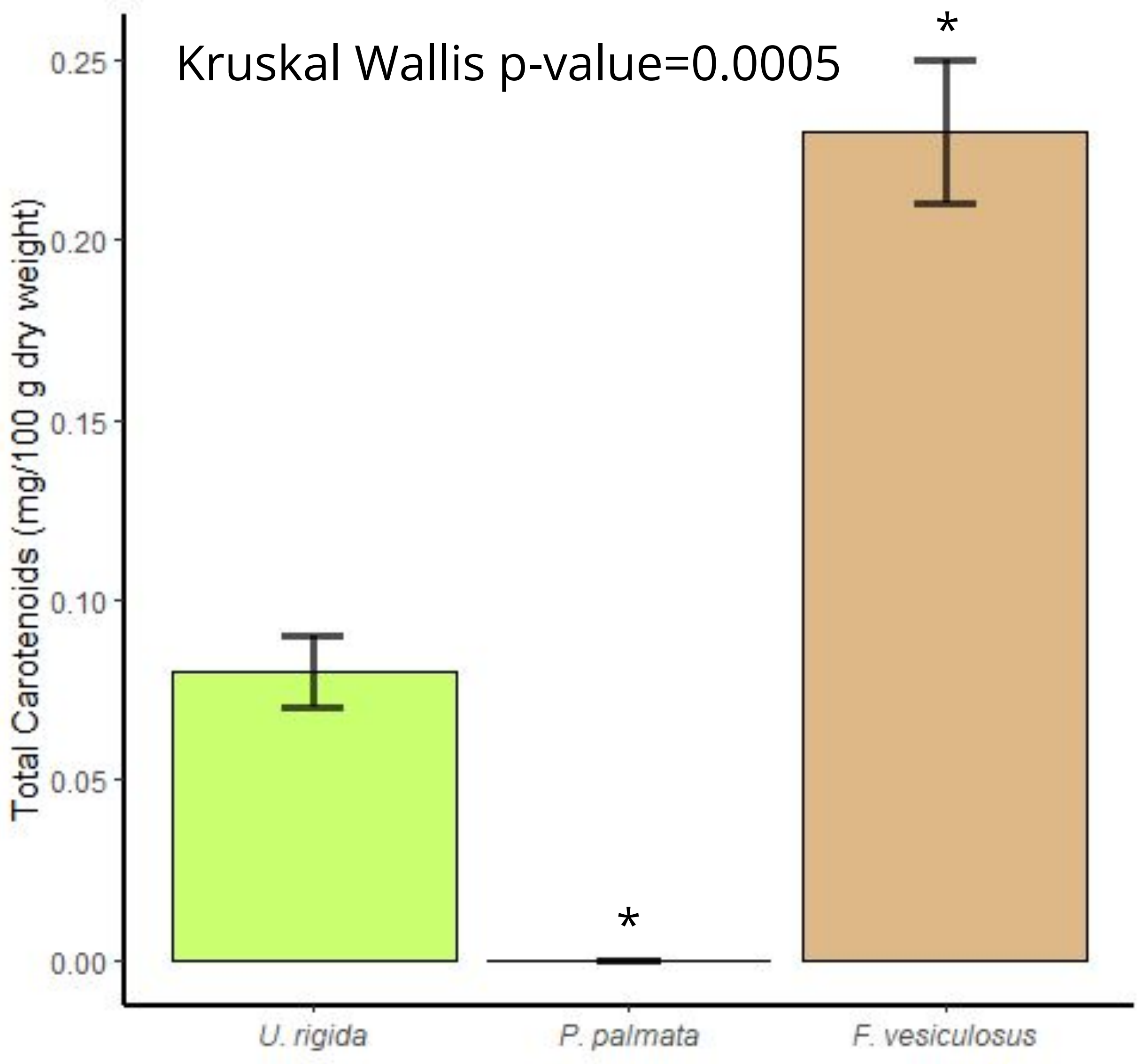
Biomass mixed with acetone-hexane (4:6), filtered




**Dunn (BONFERRONI) p-value**
U. rigida vs P. palmata
**0.0003**

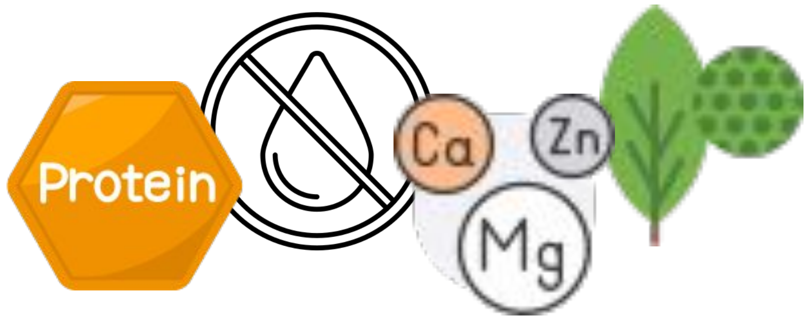



**Dunn (BONFERRONI) p-value**
U. rigida vs P. palmata
**0.0001**




**Dunn (BONFERRONI) p-value**
P. palmata vs F. vesiculosus
**0.0001**

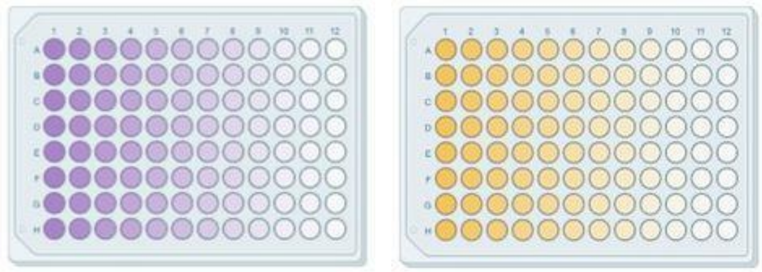
# GRAPHICAL ABSTRACT:




1. Nutricional profile

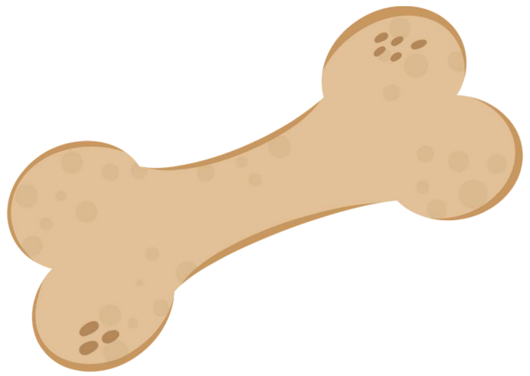

2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme

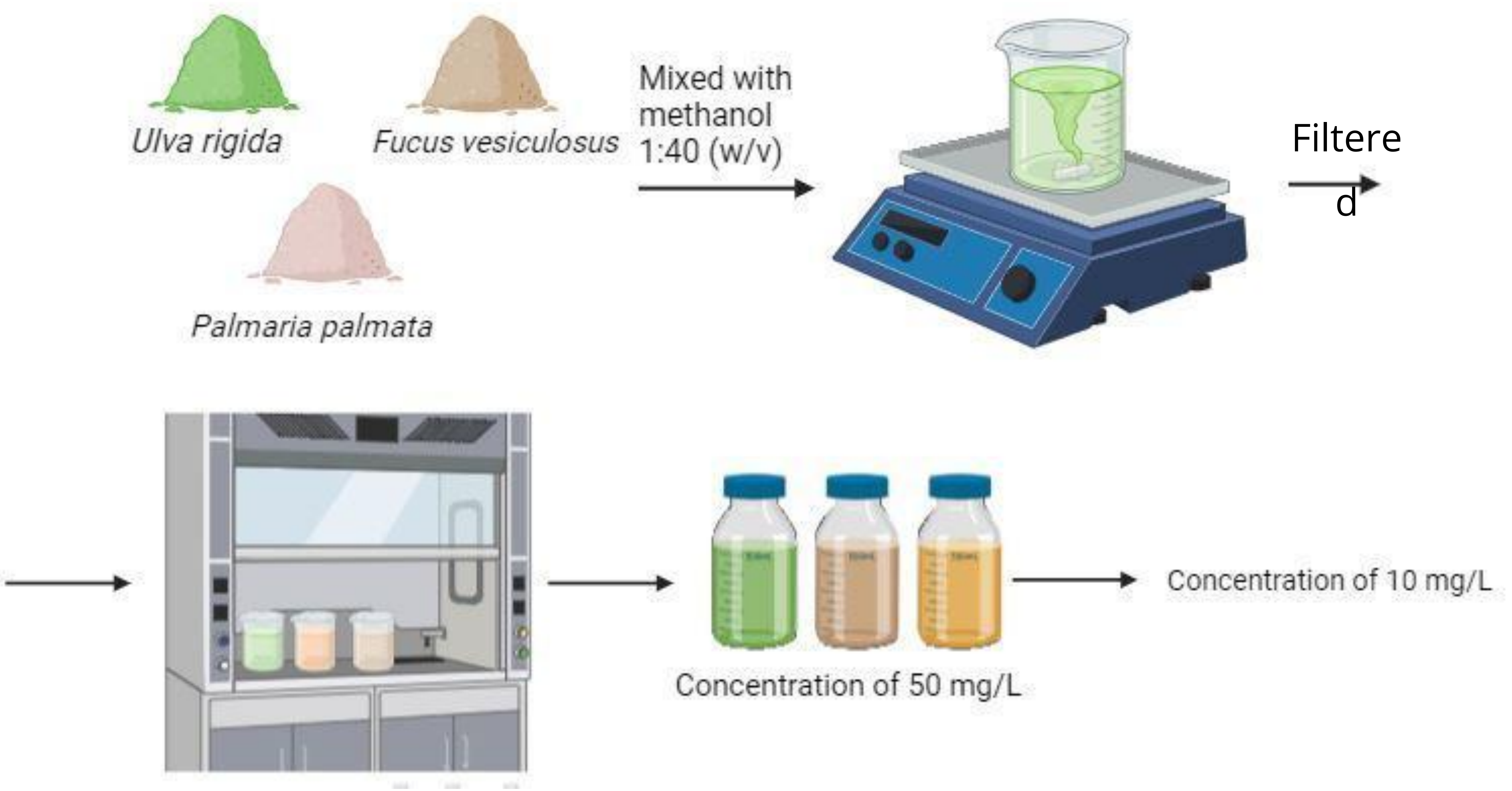


6. Cookie preparation

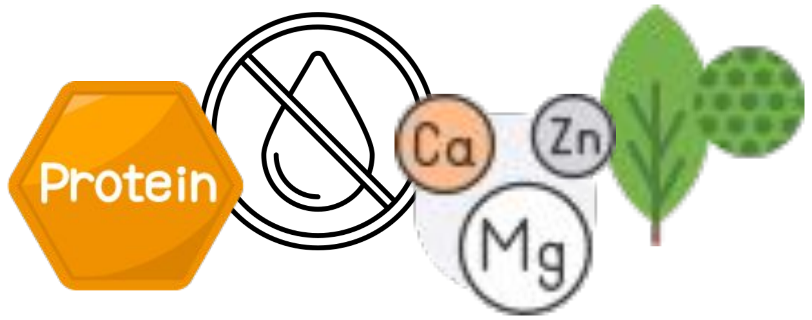
7. Snack acceptance


8. Market survey

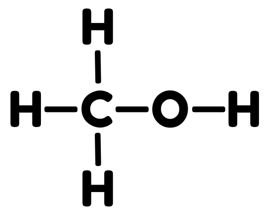
# EXTRACTION


Ulva rigida
Fucus vesiculosus
Palmaria palmata
Mixed with methanol 1:40 (w/v)
Filtere
d
Concentration of 50 mg/L
Concentration of 10 mg/L

# GRAPHICAL ABSTRACT:

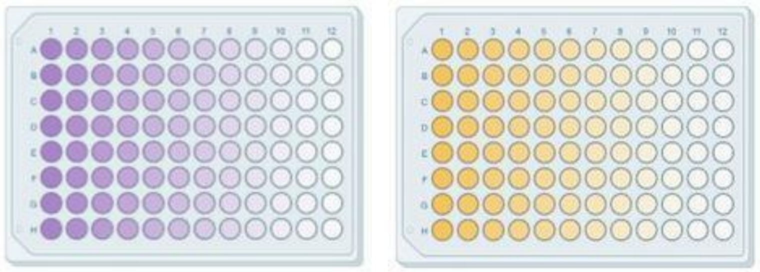



1. Nutricional profile



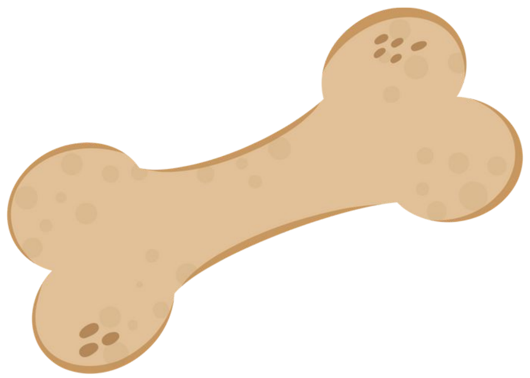
2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme



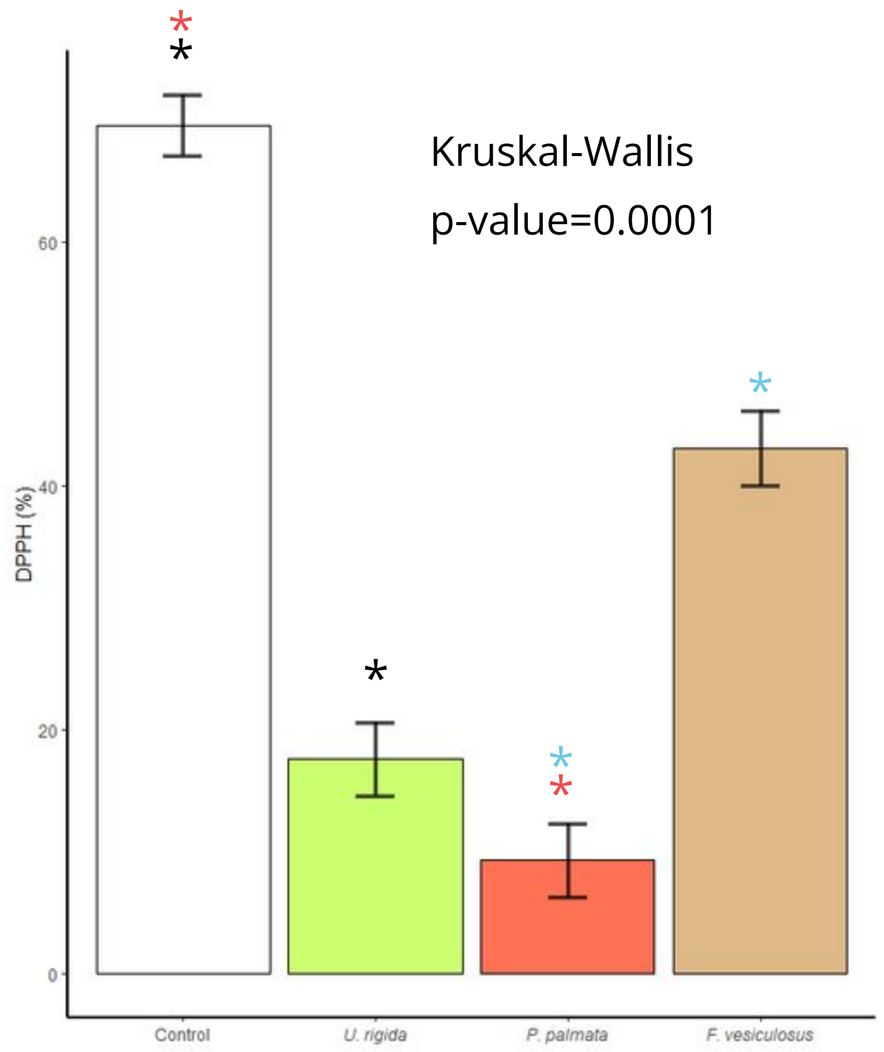
6. Cookie preparation

7. Snack acceptance


8. Market survey

# ANTIOXIDANT ACTIVITY - DPPH AND ABTS

DPPH: Method described by Brand-Williams et al. (1995) adapted to 96-well microplates (Moreno et al., 2006); Control positive = BHT

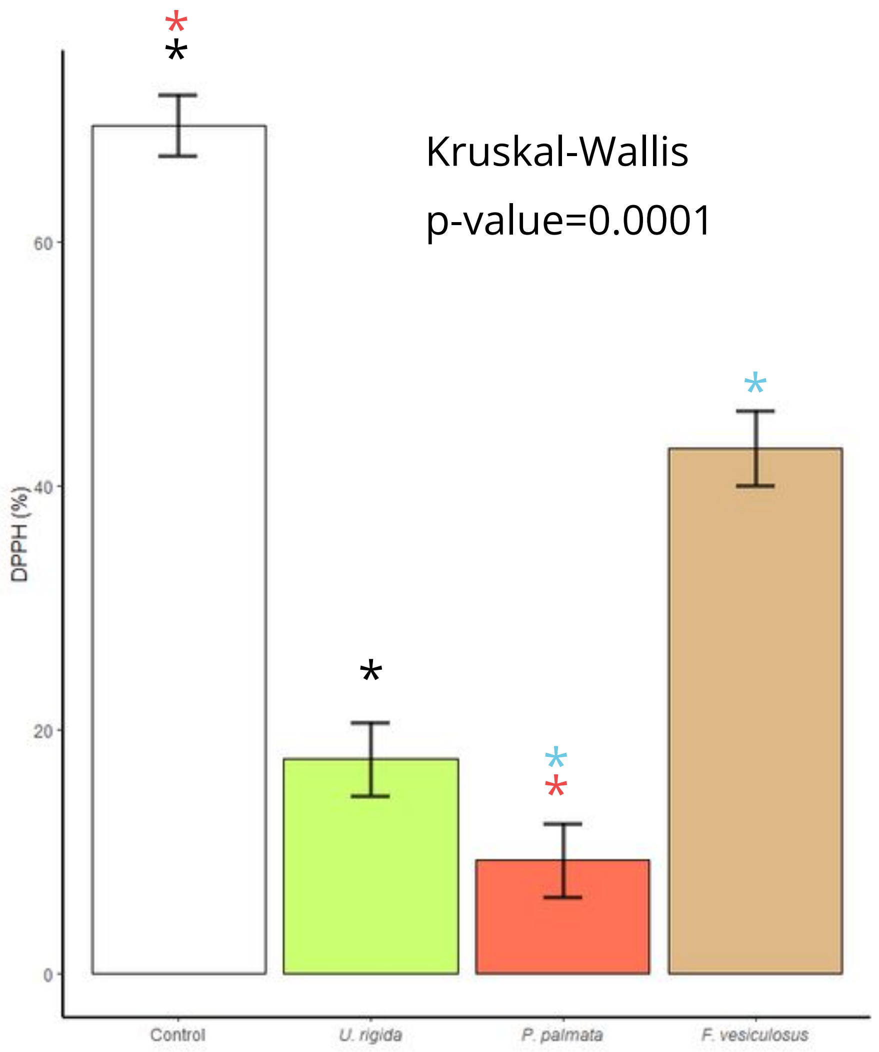



| Dunn (BONFERRONI) p-value | |
|---|---|
| Control vs U. rigida cookies | **0.008** |
| Control vs P. palmata cookies | **0.00005** |
| P. palmata cookies vs F. vesiculosus cookies | **0.01** |

# ANTIOXIDANT ACTIVITY - DPPH AND ABTS

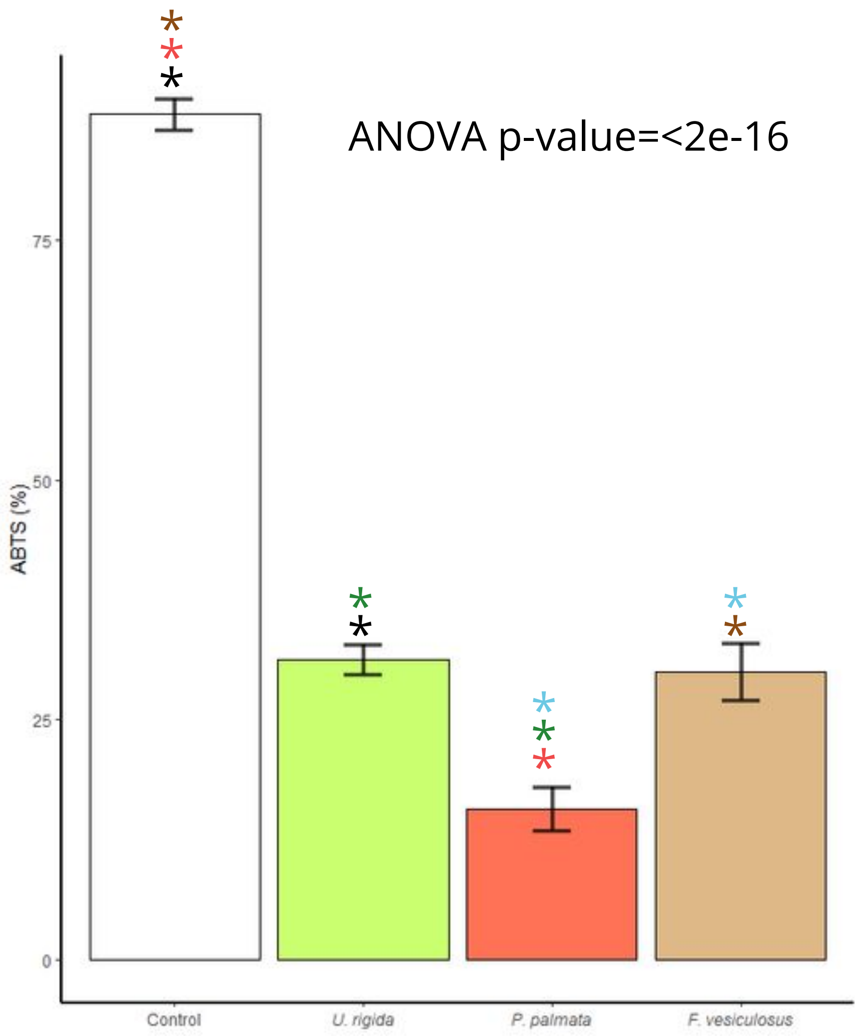
DPPH: Method described by Brand-Williams et al. (1995) adapted to 96-well microplates (Moreno et al., 2006); Control positive = BHT

ABTS: Method described by Re et al. (1999); Control positive = BHT




**Dunn (BONFERRONI) p-value**

| | |
|---|---|
| Control vs U. rigida cookies | **0.008** |
| Control vs P. palmata cookies | **0.00005** |
| P. palmata cookies vs F. vesiculosus cookies | **0.01** |

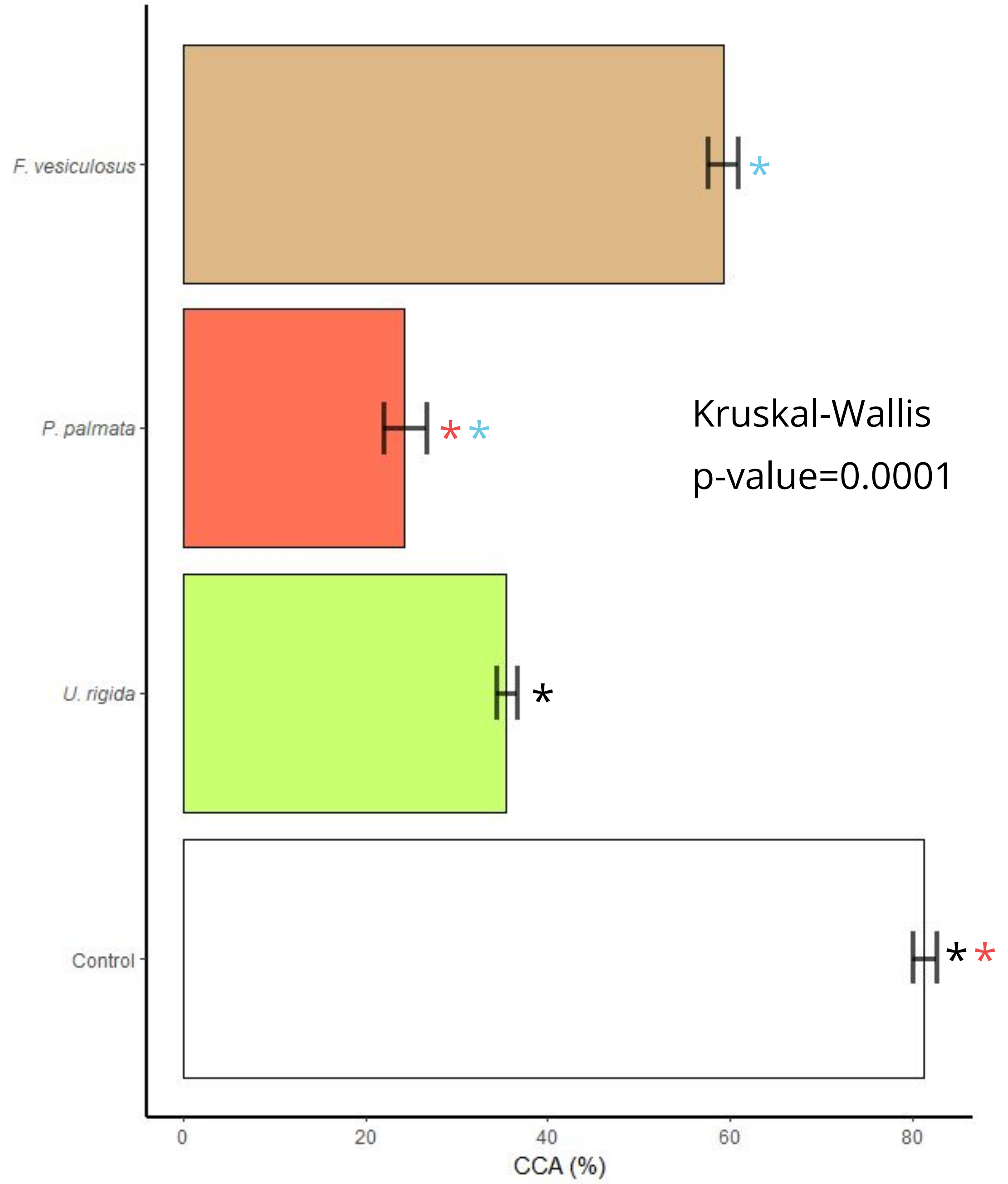



**Tukey p-value**

| | |
|---|---|
| Control vs U. rigida cookies | **~0** |
| Control vs P. palmata cookies | **~0** |
| Control vs F. vesiculosus cookies | **~0** |
| U. rigida cookies vs P. palmata cookies | **~0** |
| P. palmata cookies vs F. vesiculosus cookies | |

# ANTIOXIDANT ACTIVITY - CCA

Method described by Megías et al. (2009). Control positive = EDTA




Kruskal-Wallis
p-value=0.0001

**Dunn (BONFERRONI) p-value**

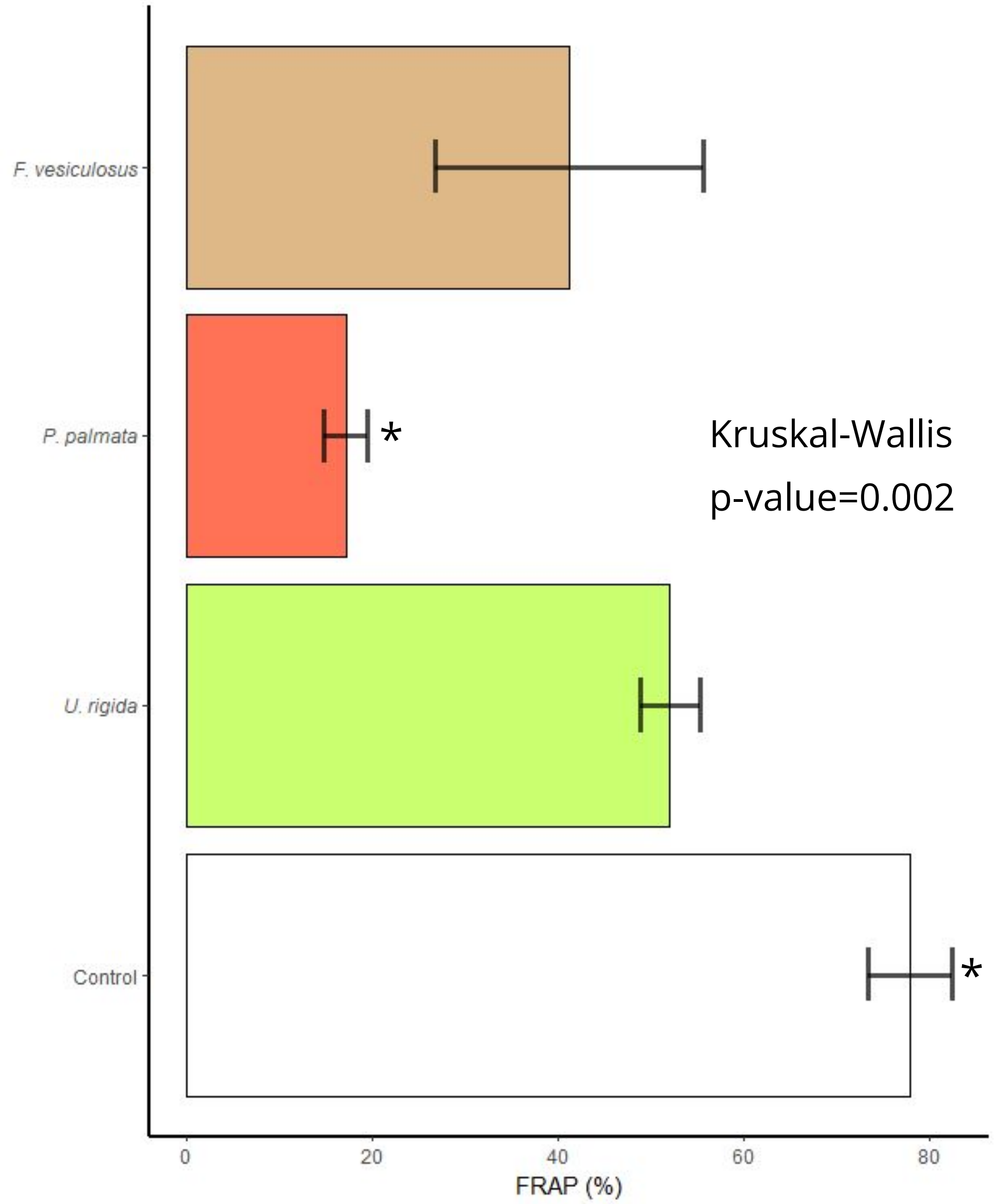
Control vs U. rigida cookies **0.009**

Control vs P. palmata cookies **~0**

P. palmata cookies vs F. vesiculosus cookies **0.009**

# ANTIOXIDANT ACTIVITY - FRAP

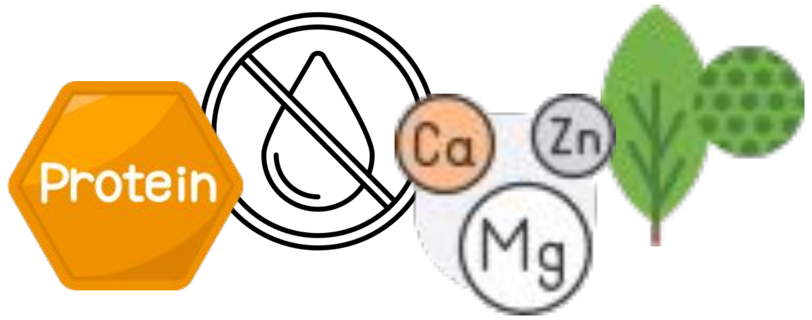
Method described by Oyaizu (1986), and modified by Megías et al. (2009); Control positive = BHT.



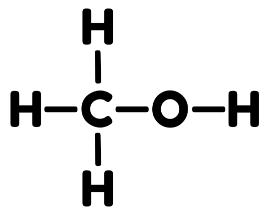

Kruskal-Wallis
p-value=0.002

| **Dunn (BONFERRONI) p-value** | |
|---|---|
| Control vs P. palmata cookies | **~0** |

GRAPHICAL ABSTRACT:

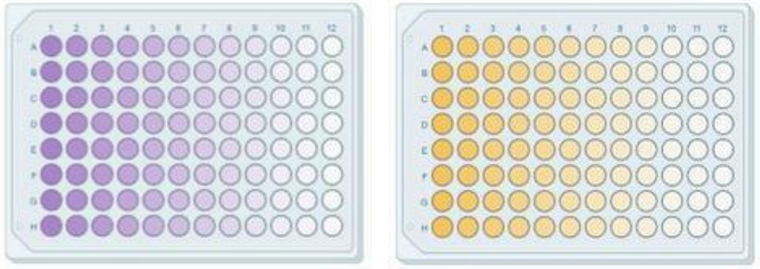



1. Nutricional profile



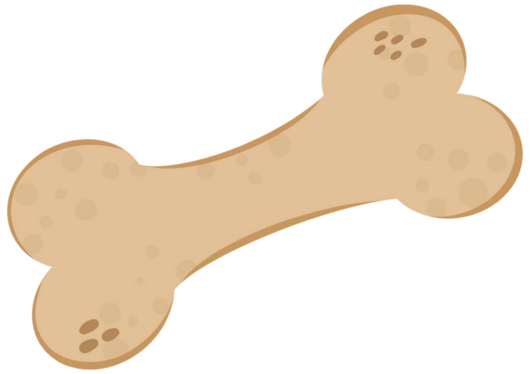

2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme



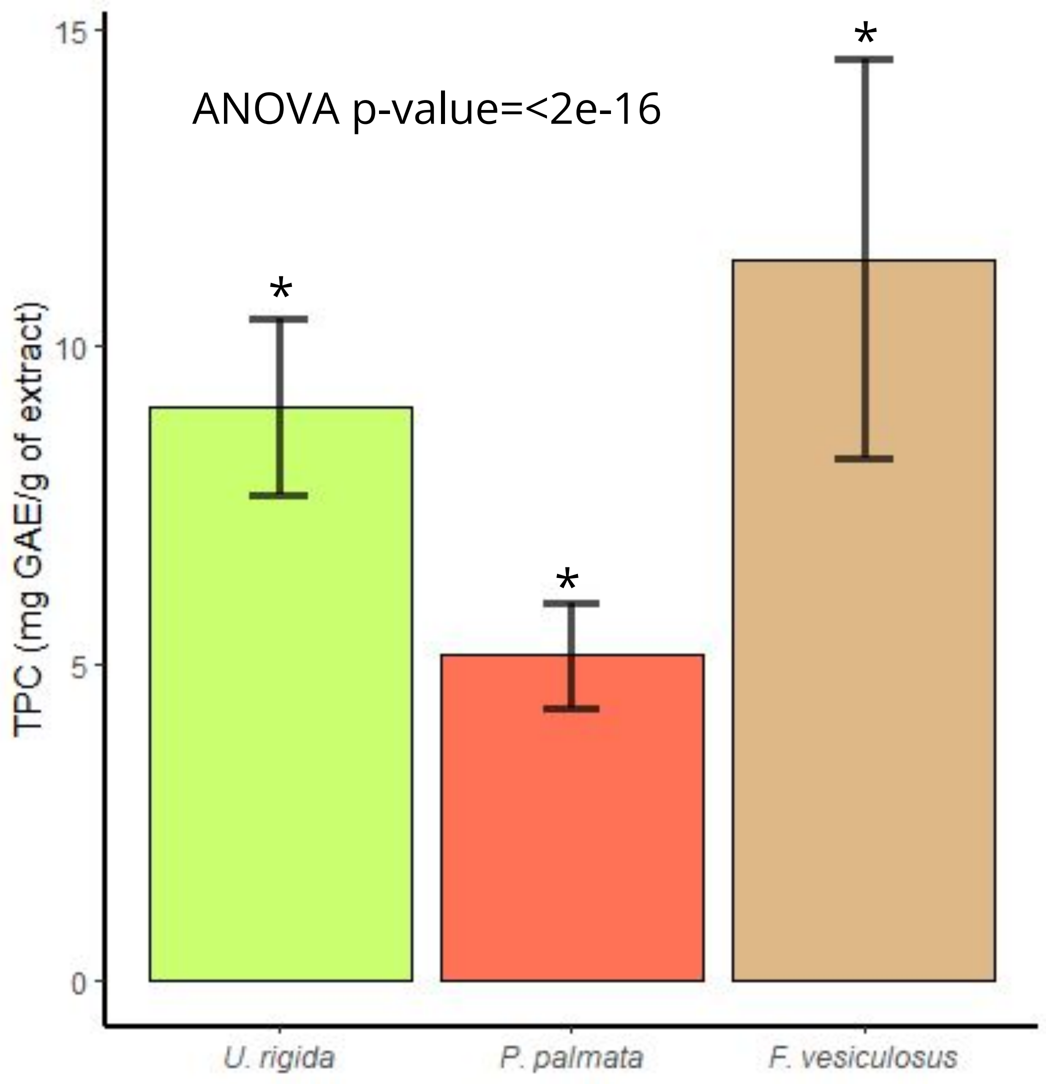
6. Cookie preparation

7. Snack acceptance


8. Market survey

# TOTAL CONTENTS OF PHENOLICS (TPC) AND FLAVONOIDS (TFC)

Phenolics (TPC): the Folin-Ciocalteu assay (Velioglu et al., 1998)




**Tukey p-value**

U. rigida vs P. palmata

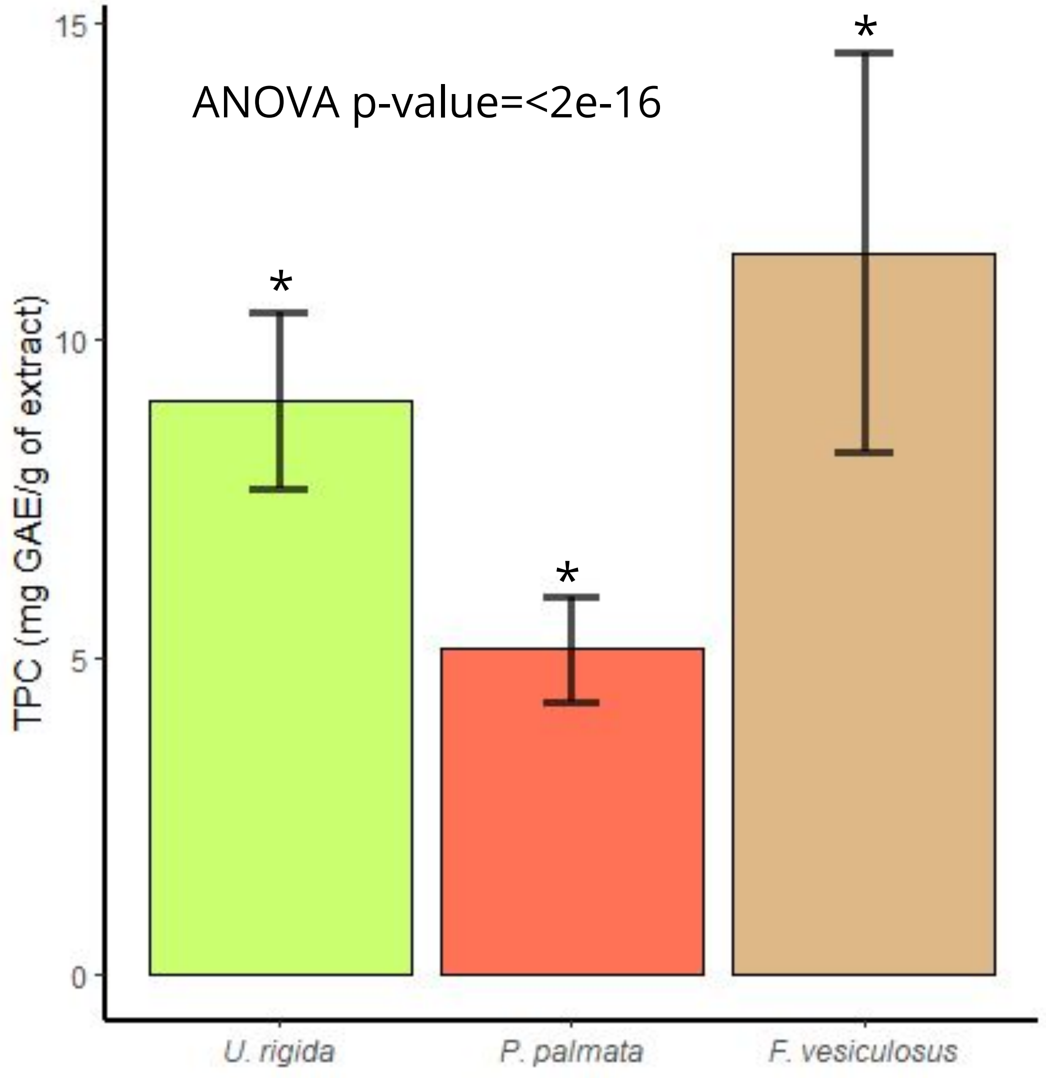
**~0**

U. rigida vs F. vesiculosus

# TOTAL CONTENTS OF PHENOLICS (TPC) AND FLAVONOIDS (TFC)

Phenolics (TPC): the Folin-Ciocalteu assay (Velioglu et al., 1998)

Flavonoids (TFC): AlCl3 colorimetric assay adapted to 96-well microplates

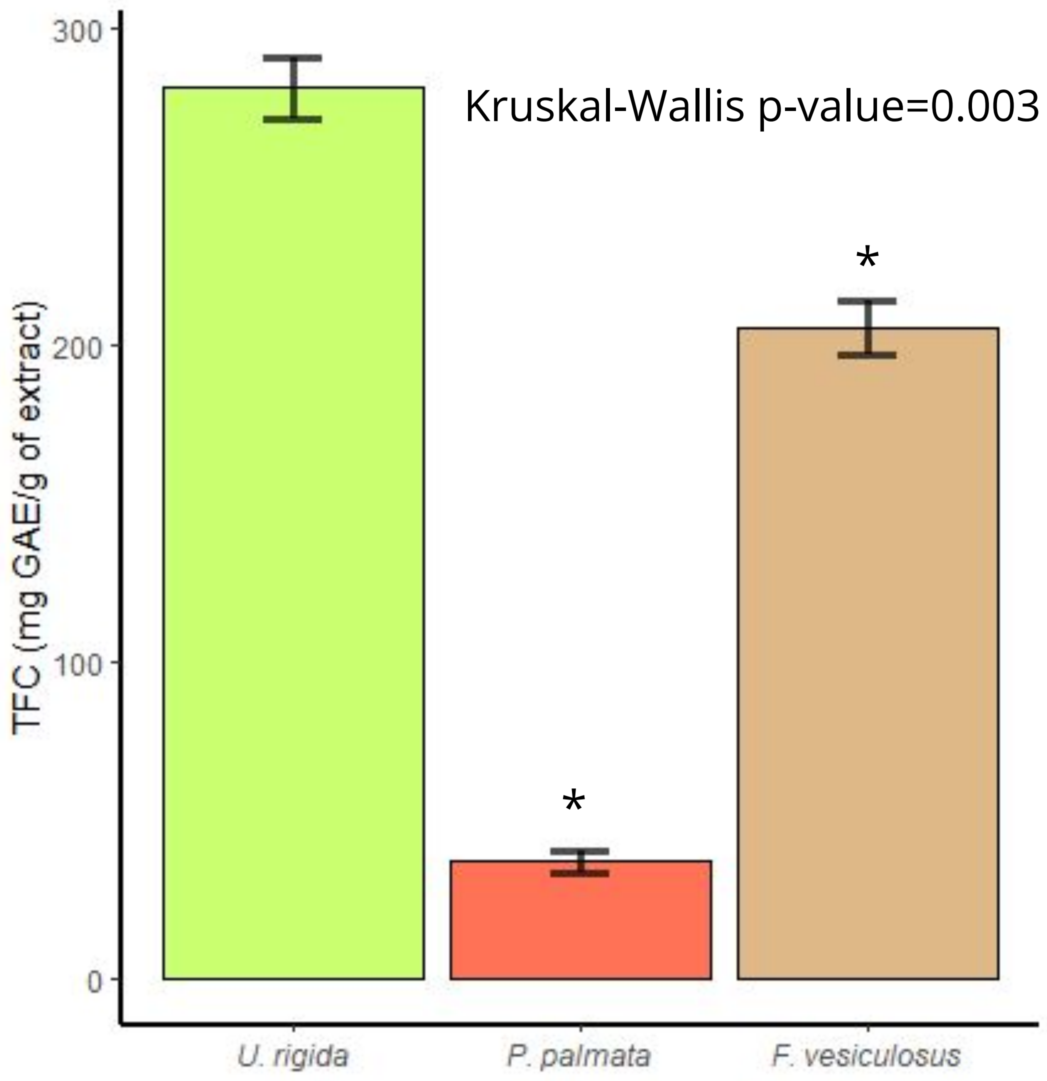



**Tukey p-value**

U. rigida vs P. palmata

**~0**

U. rigida vs F. vesiculosus

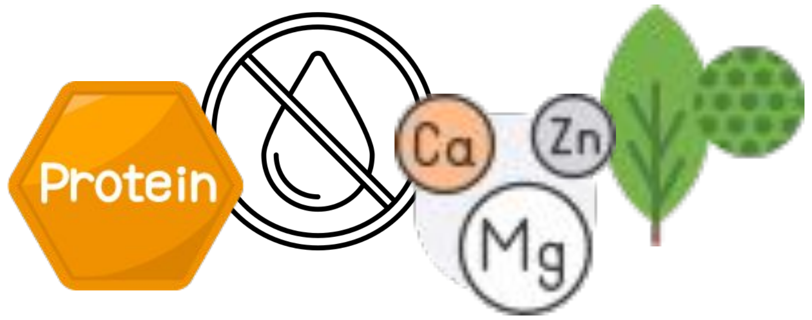



**Dunn (BONFERRONI) p-value**

P. palmata vs F. vesiculosus

**0.001**

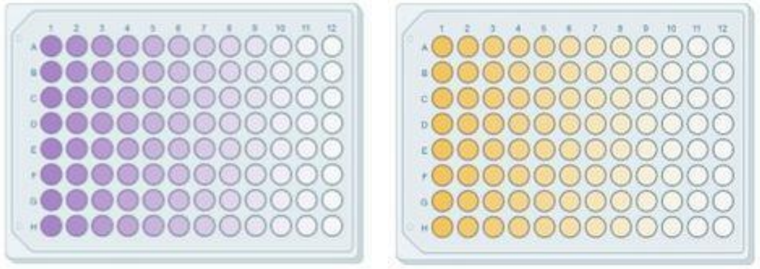
## GRAPHICAL ABSTRACT:




1. Nutricional profile

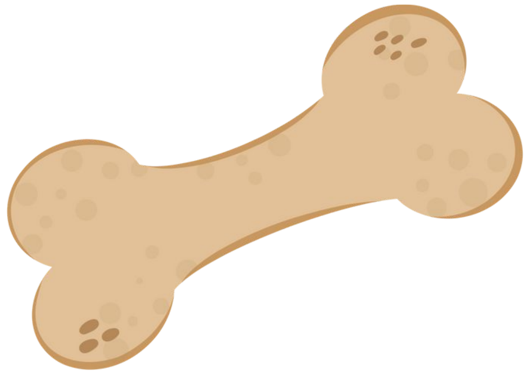

2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme



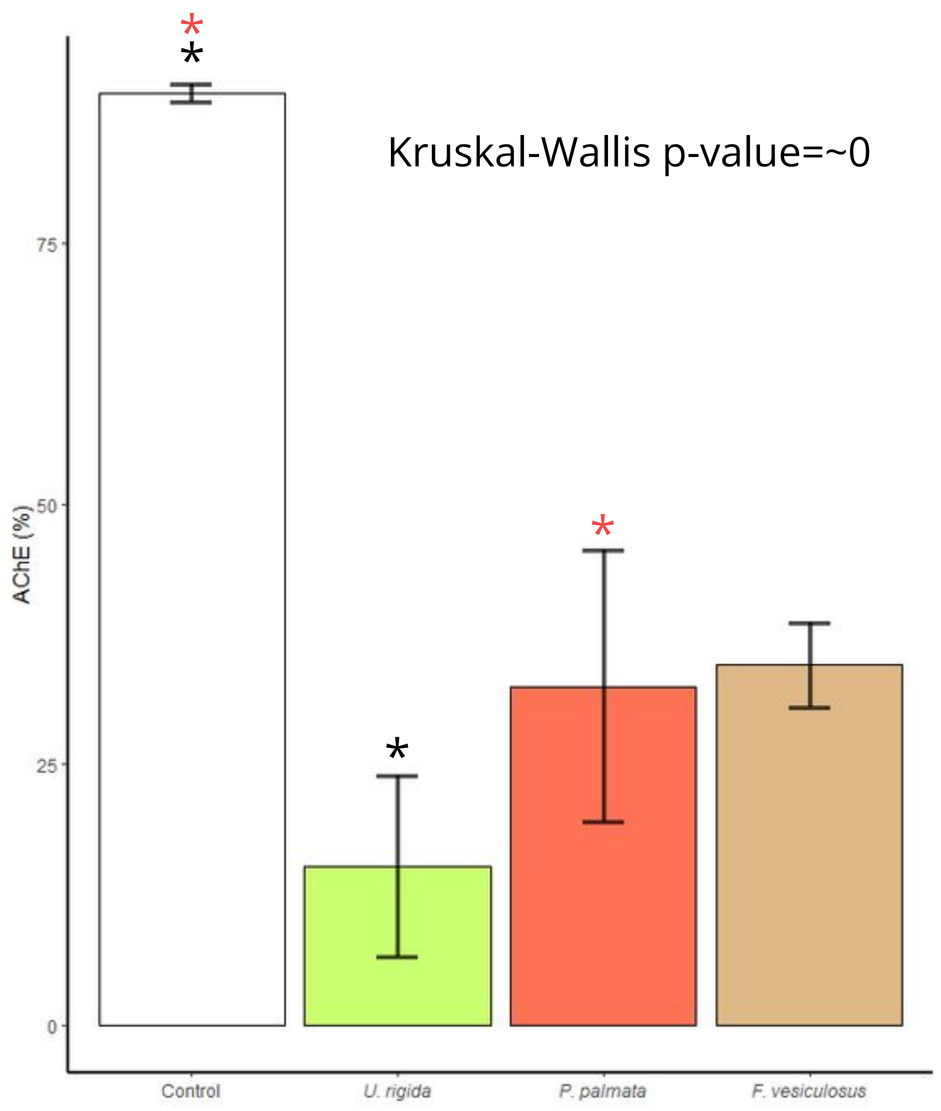
6. Cookie preparation

7. Snack acceptance


8. Market survey

# ENZYME

Method described by Ellman et al. (1961). Control positive = Galantamine



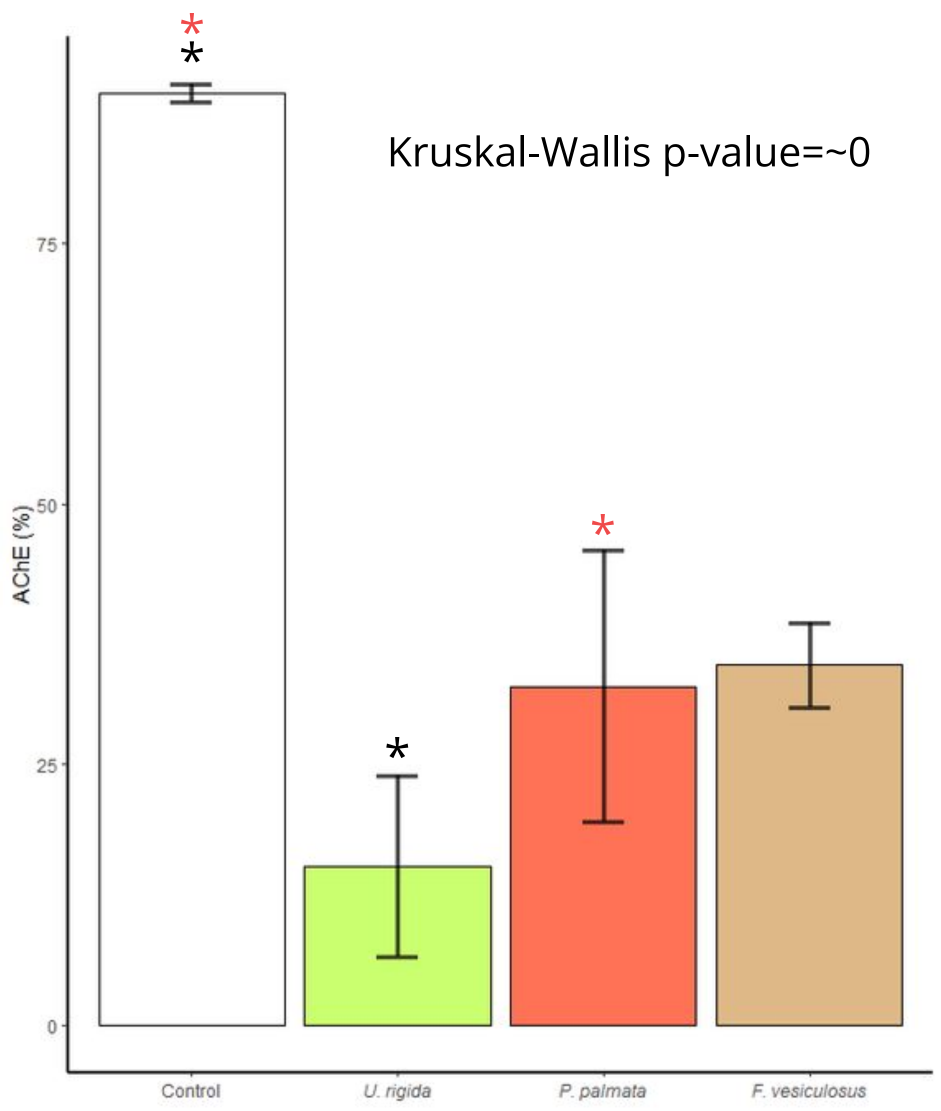

**Dunn (BONFERRONI) p-value**

Control vs U. rigida cookies **0.0008**

Control vs P. palmata cookies **0.008**

# ENZYME

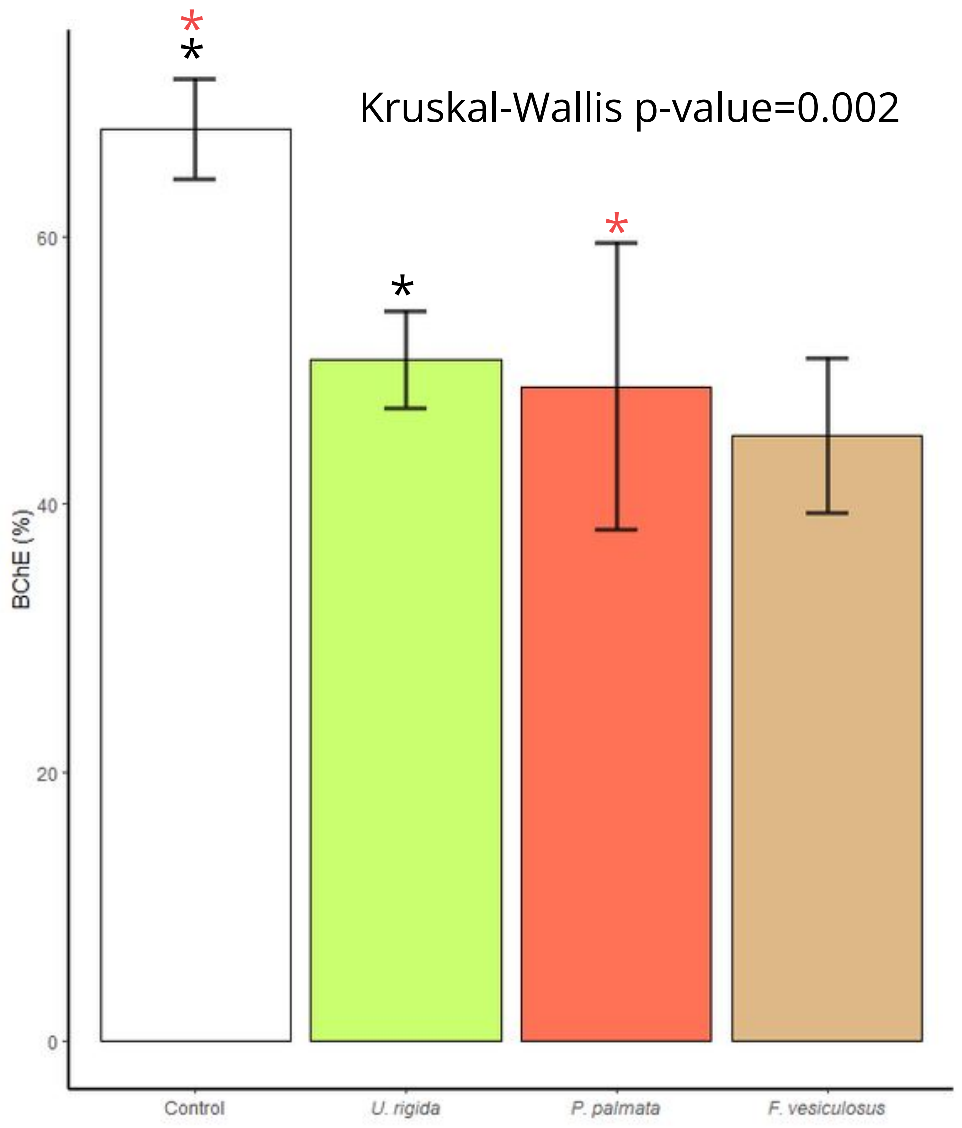
Method described by Ellman et al. (1961). Control positive = Galantamine




**Dunn (BONFERRONI) p-value**

Control vs U. rigida cookies **0.0008**

Control vs P. palmata cookies **0.008**

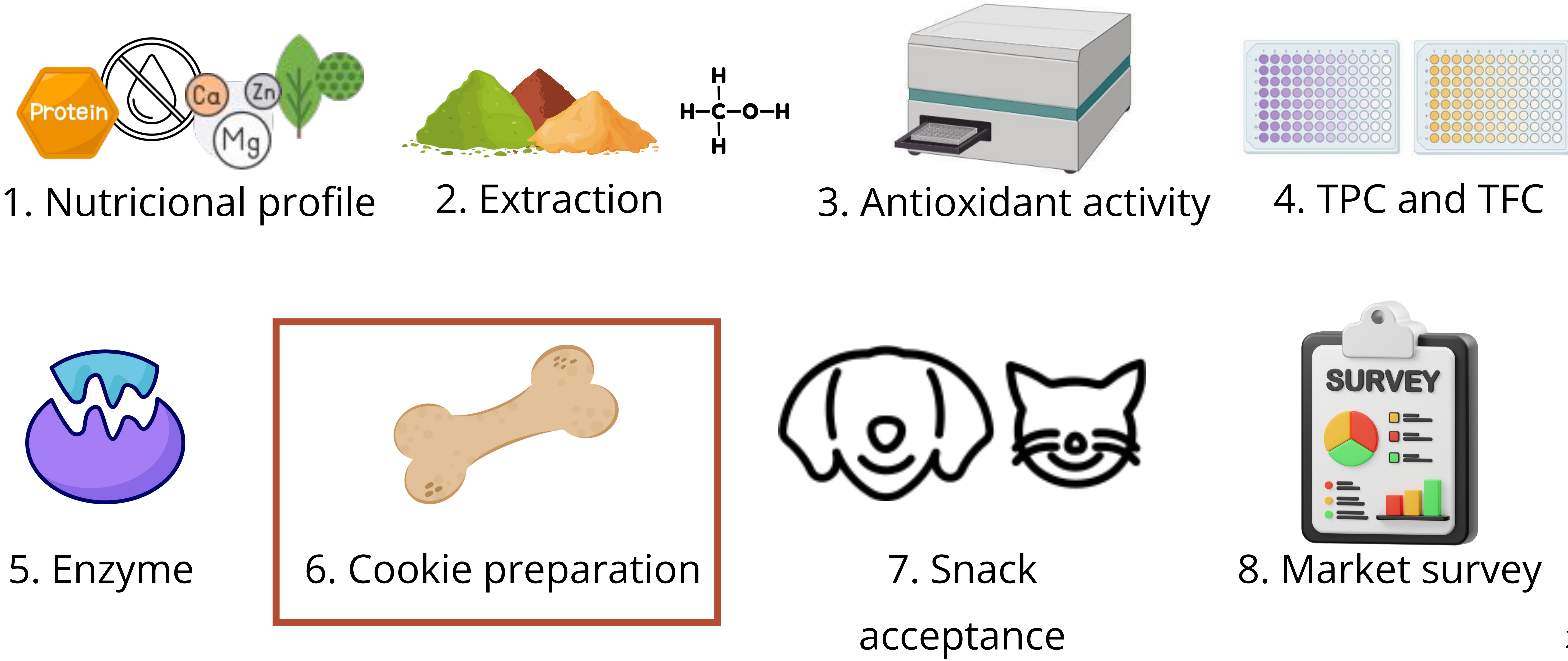



**Dunn (BONFERRONI) p-value**

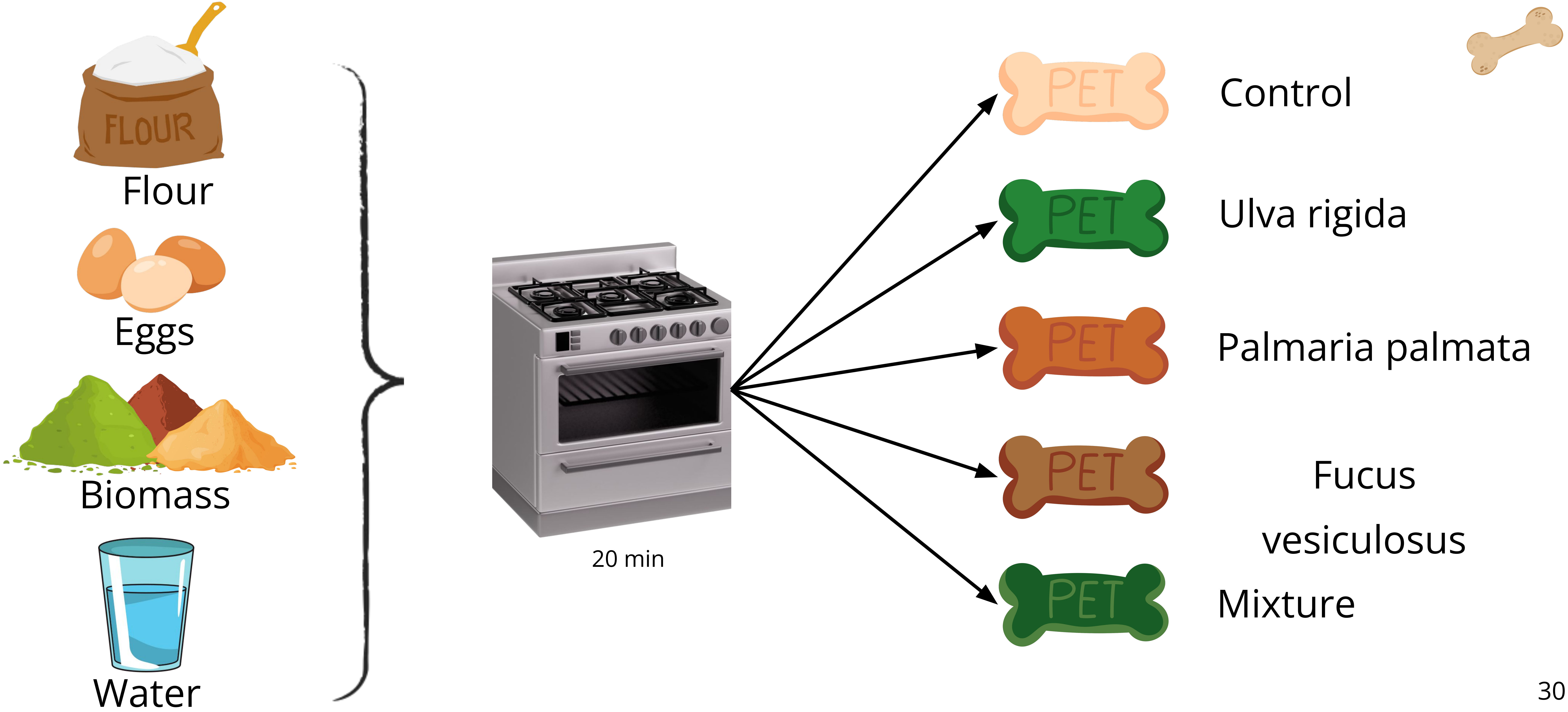
Control vs U. rigida cookies **0.0007**

Control vs P. palmata cookies **0.02**

# GRAPHICAL ABSTRACT:



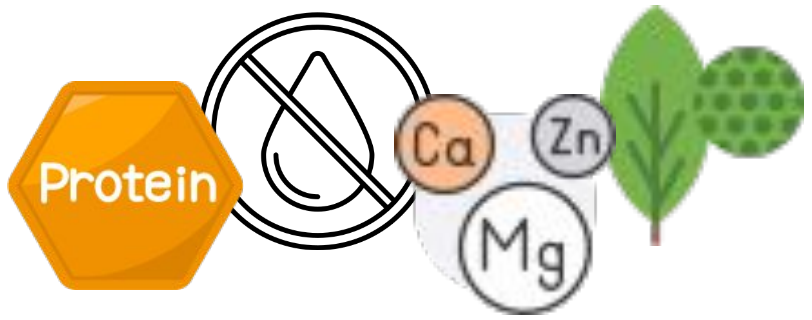
# COOKIE PREPARATION



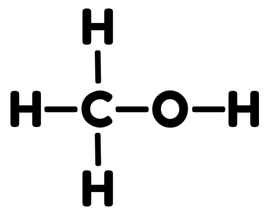
# COOKIE PREPARATION


Control | Ulva rigida | Palmaria palmata | Fucus vesiculosus | Mixture

# GRAPHICAL ABSTRACT:

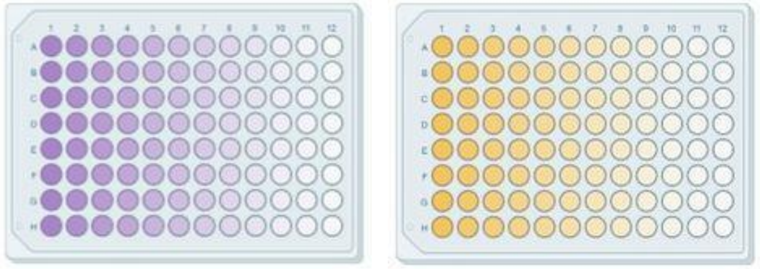



1. Nutricional profile



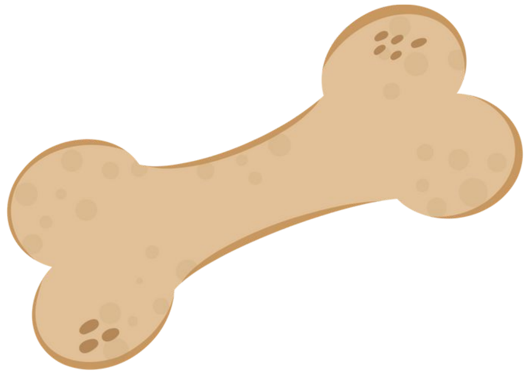
2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme

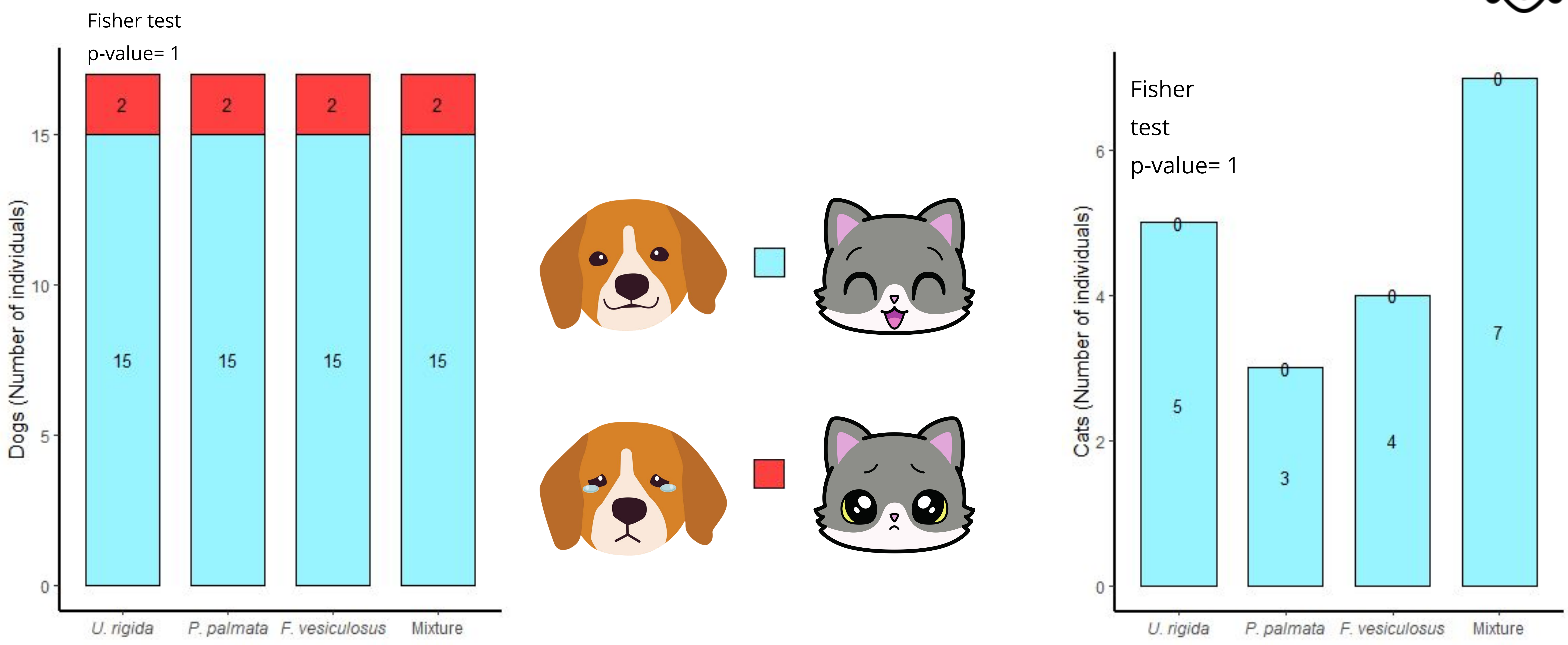


6. Cookie preparation

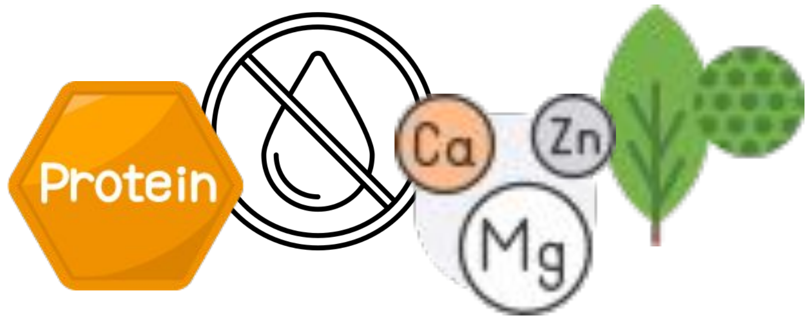
7. Snack acceptance


8. Market survey

# SNACK ACCEPTANCE



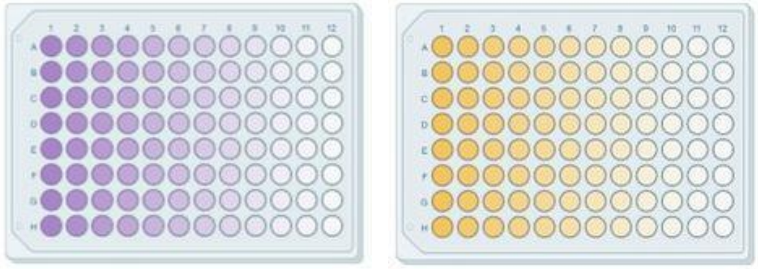
# GRAPHICAL ABSTRACT:




1. Nutricional profile

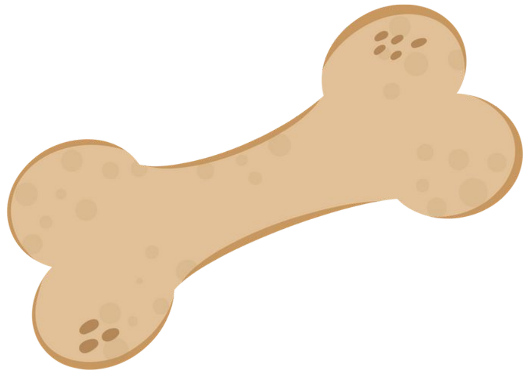
2. Extraction

3. Antioxidant activity



4. TPC and TFC

5. Enzyme



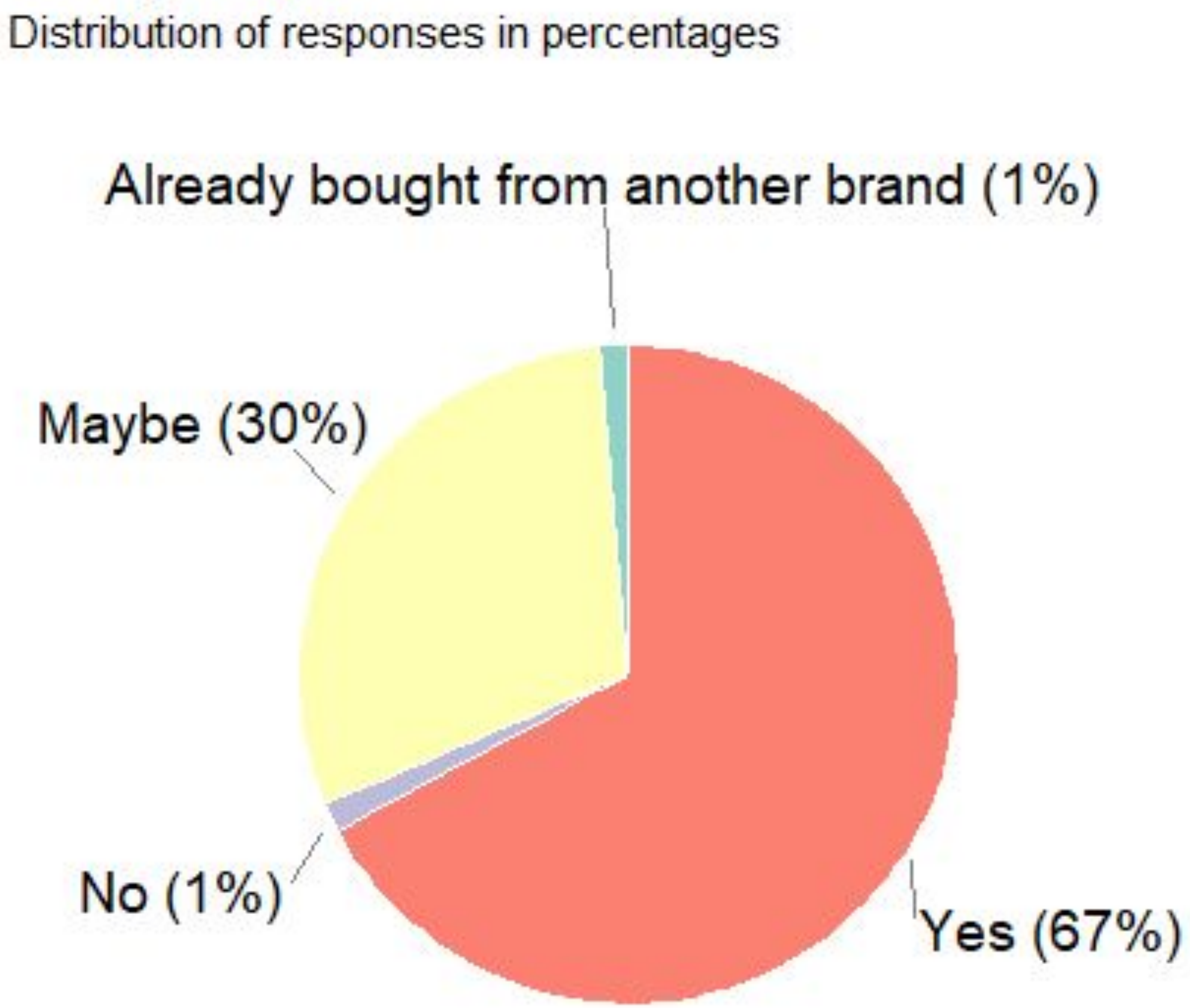
6. Cookie preparation

7. Snack acceptance

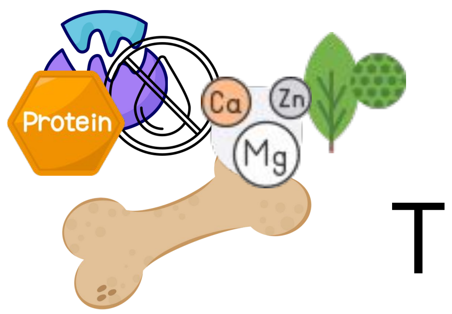

8. Market survey

Bake.my.dog.happy

# Would you buy biscuits made from seaweed?



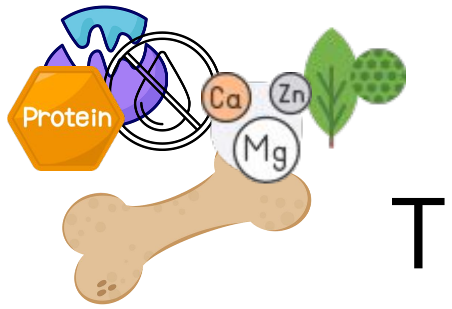
# CONCLUSIONS:




- **Cientific**

The algae and cookies have shown high antioxidant potential, enzyme inhibitory activity and chelating properties.

# CONCLUSIONS:



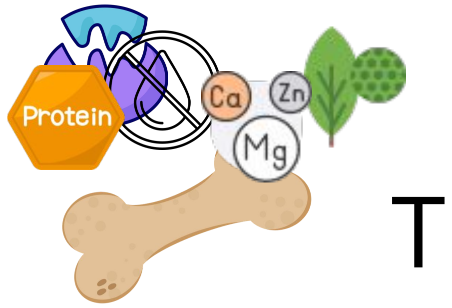

- **Cientific**

The algae and cookies have shown high antioxidant potential, enzyme inhibitory activity and chelating properties.

- **Acceptance**

Dogs and cats showed great acceptance and interest.

# CONCLUSIONS:




- **Cientific**

The algae and cookies have shown high antioxidant potential, enzyme inhibitory activity and chelating properties.

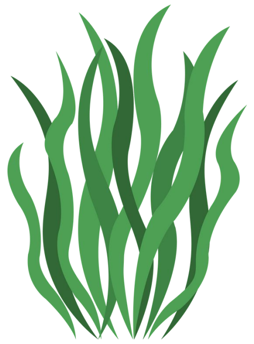
- **Acceptance**

Dogs and cats showed great acceptance and interest.


- **Market**

High consumer interest.

# FUTURE PERSPECTIVES



- **Expansion of Algae Varieties:**

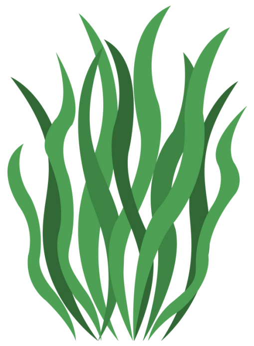
**Spirulina:** High in proteins, vitamins (B12), minerals, antioxidants, and essential fatty acids.

**Nori:** Good source of proteins, fiber, vitamins (A, C, and B12), and minerals like iodine and iron.



(Pereira, 2011; Kasimala et al., 2015)

# FUTURE PERSPECTIVES



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**Spirulina:** High in proteins, vitamins (B12), minerals, antioxidants, and essential fatty acids.

**Nori:** Good source of proteins, fiber, vitamins (A, C, and B12), and minerals like iodine and iron.

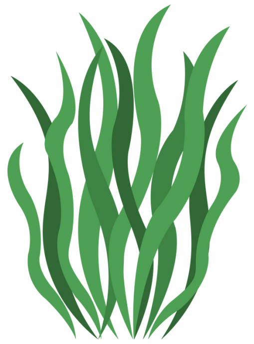
- **Flavor and Texture Optimization:**

Use fresh algae to enhance organoleptic properties.



(Pereira, 2011; Kasimala et al., 2015)

# FUTURE PERSPECTIVES



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Use fresh algae to enhance organoleptic properties.

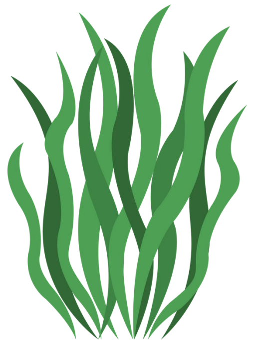
- **Preference Testing:**

Conduct preference tests by offering different cookie pieces and recording which ones the pets choose.



(Pereira, 2011; Kasimala et al., 2015)

# FUTURE PERSPECTIVES



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**Nori:** Good source of proteins, fiber, vitamins (A, C, and B12), and minerals like iodine and iron.

- **Flavor and Texture Optimization:**

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- **Preference Testing:**

Conduct preference tests by offering different cookie pieces and recording which ones the pets choose.

- **Improving Acceptance Process:**



(Pereira, 2011; Kasimala et al., 2015)

# THANK

IMBRSea

Erasmus Mundus

UAlg
UNIVERSIDADE DO ALGARVE

BAKE MY DOG HAPPY

CROAF
CENTRO DE RECOLHA OFICIAL DE ANIMAIS DE FARO

PET

francisco.nomdedeu.martinez@imbrsea.eu

# REFERENCES

# SUPLEMENTARY MATERIAL

PET

## PROTEINS

**p value Biomass**

| | |
|---|---|
| U. rigida vs P. palmata | 0.2 |
| U. rigida vs F. vesiculosus | **0.0003** |
| P. palmata vs F. vesiculosus | **0.00001** |

**p value Biomass vs Cookies**

| | |
|---|---|
| U. rigida vs Control | 0.03 |
| U. rigida vs U. rigida cookies | 0.18 |
| U. rigida vs P. palmata cookies | 1 |
| U. rigida vs F. vesiculosus cookies | 0.64 |
| U. rigida vs Mixture | **0.002** |
| P. palmata vs Control | **0.003** |
| P. palmata vs U. rigida cookies | 0.03 |
| P. palmata vs P. palmata cookies | 0.06 |
| P. palmata vs F. vesiculosus cookies | 0.13 |
| P. palmata vs Mixture | **0.0001** |
| F. vesiculosus vs Control | 1 |
| F. vesiculosus vs U. rigida cookies | 1 |
| F. vesiculosus vs P. palmata cookies | 1 |
| F. vesiculosus vs F. vesiculosus cookies | 1 |
| F. vesiculosus vs Mixture | 0.29 |

## SUGARS

**p value Biomass**

| | |
|---|---|
| U. rigida vs P. palmata | 1 |
| U. rigida vs F. vesiculosus | **0.02** |
| P. palmata vs F. vesiculosus | 0.04 |

**p value cookies**

| | |
|---|---|
| Control vs U. rigida cookies | 1 |
| Control vs P. palmata cookies | 0.1 |
| Control vs F. vesiculosus cookies | **0.0024** |
| Control vs Mixture | 1 |
| U. rigida cookies vs P. palmata cookies | **0.004** |
| U. rigida cookies vs F. vesiculosus cookies | **~0** |
| U. rigida cookies vs Mixture | 0.26 |
| P. palmata cookies vs F. vesiculosus cookies | 1 |
| P. palmata cookies vs Mixture | 0.79 |
| F. vesiculosus vs Mixture | 0.05 |

**p value Biomass vs cookies**

| | |
|---|---|
| U. rigida vs Control | 0.14 |
| U. rigida vs U. rigida cookies | 0.75 |
| U. rigida vs P. palmata cookies | **0.0007** |
| U. rigida vs F. vesiculosus cookies | **~0** |
| U. rigida vs Mixture | **0.2** |
| P. palmata vs Control | 0.16 |
| P. palmata vs U. rigida cookies | 0.87 |
| P. palmata vs P. palmata cookies | **0.001** |
| P. palmata vs F. vesiculosus cookies | **~0** |
| P. palmata vs Mixture | 0.03 |
| F. vesiculosus vs Control | 1 |
| F. vesiculosus vs U. rigida cookies | 1 |
| F. vesiculosus vs P. palmata cookies | 0.03 |
| F. vesiculosus vs F. vesiculosus cookies | **0.002** |
| F. vesiculosus vs Mixture | 1 |

# SUPLEMENTARY MATERIAL

## MOISTURE

**p value Biomass**

U. rigida vs P. palmata **0.00001**

U. rigida vs F. vesiculosus **0.001**

P. palmata vs F. vesiculosus **0.0004**

**p value Cookies**

Control vs U. rigida cookies **0.00003**

Control vs P. palmata cookies **0.002**

Control vs F. vesiculosus cookies 0.77

Control vs Mixture 0.046

U. rigida cookies vs P. palmata cookies **0.02**

U. rigida cookies vs F. vesiculosus cookies **0.00008**

U. rigida cookies vs Mixture **0.001**

P. palmata cookies vs F. vesiculosus cookies **0.01**

P. palmata cookies vs Mixture 0.33

F. vesiculosus vs Mixture 0.27

**p value Cookies vs Biomass**

U. rigida vs Control 0.06

U. rigida vs U. rigida cookies 1

U. rigida vs P. palmata cookies 1

U. rigida vs F. vesiculosus cookies 0.13

U. rigida vs Mixture 0.79

P. palmata vs Control 1

P. palmata vs U. rigida cookies 0.46

P. palmata vs P. palmata cookies 1

P. palmata vs F. vesiculosus cookies 1

P. palmata vs Mixture 1

F. vesiculosus vs Control 0.39

F. vesiculosus vs U. rigida cookies 1

F. vesiculosus vs P. palmata cookies 1

F. vesiculosus vs F. vesiculosus cookies 0.79

F. vesiculosus vs Mixture 1

## ASH

**p value Biomass**

U. rigida vs P. palmata 0.17

U. rigida vs F. vesiculosus **0.002**

P. palmata vs F. vesiculosus **0.0004**

**p value cookies**

Control vs U. rigida cookies **0.00001**

Control vs P. palmata cookies **~0**

Control vs F. vesiculosus cookies **~0**

Control vs Mixture **~0**

U. rigida cookies vs P. palmata cookies **~0**

U. rigida cookies vs F. vesiculosus cookies **~0**

U. rigida cookies vs Mixture **0.000004**

P. palmata cookies vs F. vesiculosus cookies **0.00009**

P. palmata cookies vs Mixture **0.0002**

F. vesiculosus vs Mixture **0.0000002**

**p value Biomass vs cookies**

U. rigida vs Control **~0**

U. rigida vs U. rigida cookies **~0**

U. rigida vs P. palmata cookies **~0**

U. rigida vs F. vesiculosus cookies **~0**

U. rigida vs Mixture **~0**

P. palmata vs Control **~0**

P. palmata vs U. rigida cookies **~0**

P. palmata vs P. palmata cookies **~0**

P. palmata vs F. vesiculosus cookies **~0**

P. palmata vs Mixture **~0**

# SUPLEMENTARY MATERIAL

## LIPIDS

| p value cookies | |
|---|---|
| Control vs U. rigida cookies | 0.14 |
| Control vs P. palmata cookies | **0.009** |
| Control vs F. vesiculosus cookies | 0.34 |
| Control vs Mixture | 1 |
| U. rigida cookies vs P. palmata cookies | 1 |
| U. rigida cookies vs F. vesiculosus cookies | 1 |
| U. rigida cookies vs Mixture | 1 |
| P. palmata cookies vs F. vesiculosus cookies | 1 |
| P. palmata cookies vs Mixture | 0.22 |
| F. vesiculosus vs Mixture | 1 |

| p value biomass vs cookies | |
|---|---|
| U. rigida vs Control | 0.21 |
| U. rigida vs U. rigida cookies | 1 |
| U. rigida vs P. palmata cookies | 1 |
| U. rigida vs F. vesiculosus cookies | 1 |
| U. rigida vs Mixture | 1 |
| P. palmata vs Control | 1 |
| P. palmata vs U. rigida cookies | 1 |
| P. palmata vs P. palmata cookies | 0.45 |
| P. palmata vs F. vesiculosus cookies | 1 |
| P. palmata vs Mixture | 1 |
| F. vesiculosus vs Control | 0.04 |
| F. vesiculosus vs U. rigida cookies | 1 |
| F. vesiculosus vs P. palmata cookies | 1 |
| F. vesiculosus vs F. vesiculosus cookies | 1 |
| F. vesiculosus vs Mixture | 0.78 |

## DPPH

| p value | |
|---|---|
| Control vs U. rigida cookies | **0.008** |
| Control vs P. palmata cookies | **0.00005** |
| Control vs F. vesiculosus cookies | 0.42 |
| U. rigida cookies vs P. palmata cookies | 0.57 |
| U. rigida cookies vs F. vesiculosus cookies | 0.36 |
| P. palmata cookies vs F. vesiculosus cookies | **0.01** |

## ABTS

| p value | |
|---|---|
| Control vs U. rigida cookies | **~0** |
| Control vs P. palmata cookies | **~0** |
| Control vs F. vesiculosus cookies | **~0** |
| U. rigida cookies vs P. palmata cookies | **~0** |
| U. rigida cookies vs F. vesiculosus cookies | 0.76 |
| P. palmata cookies vs F. vesiculosus cookies | **~0** |

## CHLOROPHYLL A

| p value | |
|---|---|
| U. rigida vs P. palmata | **0.0003** |
| U. rigida vs F. vesiculosus | 0.16 |
| P. palmata vs F. vesiculosus | 0.05 |

## CHLOROPHYLL B

| p value | |
|---|---|
| U. rigida vs P. palmata | **0.0001** |
| U. rigida vs F. vesiculosus | 0.08 |
| P. palmata vs F. vesiculosus | 0.08 |

## CAROTENOIDS

| p value | |
|---|---|
| U. rigida vs P. palmata | 0.08 |
| U. rigida vs F. vesiculosus | 0.08 |
| P. palmata vs F. vesiculosus | **0.0001** |

# SUPLEMENTARY MATERIAL

## MINERALS

| | | Biomass | | | Cookies | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Minerals** | **Ulva rigida** | **Palmaria palmata** | **Fucus vesiculosus** | **Control** | **Ulva rigida** | **Palmaria palmata** | **Fucus vesiculosus** | **Mixture** |
| **Macrominerals** | **P (mg/g)** | 7.74 ± 1.76a | 7.69 ± 1.47a | 7.97 ± 1.44a | 17.33 ± 1.1a | 14.91 ± 0.82a | 15.62 ± 0.5a | 14.24 ± 0.6a | 15.8 ± 0.52a |
| | **Ca (mg/g)** | 8.92 ± 6.31a | 8.08 ± 5.71a | 8.02 ± 5.72a | 2.32 ± 0.16a | 5.67 ± 0.18a | 5.08 ± 0.17a | 12.75 ± 0.6a | 7.31 ± 0.14a |
| | **K (mg/g)** | 87.45 ± 37.37a | 86.26 ± 35.3a | 91.1 ± 37.74a | 14.69 ± 0.73b | 19.95 ± 0.64bc | 46.92 ± 1.71d | 39.81 ± 1.94bc | 35.2 ± 0.73bc |
| | **Mg (mg/g)** | 55.45 ± 38.56a | 54.63 ± 37.3a | 57.07 ± 38.77a | 5.98 ± 0.52b | 30.88 ± 1.21ab | 8.4 ± 0.22b | 16.03 ± 0.96b | 17.91 ± 0.57b |
| | **Fe (mg/g)** | 3.51 ± 1.48 a | 3.44 ± 1.47a | 3.65 ± 1.58a | 0.15 ± 0.02a | 1.32 ± 0.03a | 0.47 ± 0.02a | 1.2 ± 0.09a | 0.95 ± 0.05a |
| **Trace elements** | **Zn (µg/g)** | 208.2 ± 171.62a | 211.03 ± 183.41a | 225.37 ± 203.68a | 160.67 ± 1.27a | 134.93 ± 3.23a | 136.2 ± 6.42a | 229.4 ± 7.94a | 165.73 ± 2.54a |
| | **Cd (µg/g)** | 7.1 ± 2.2a | 7.37 ± 2.78a | 6.67 ± 2.86a | 4.97 ± 0.24a | 4.6 ± 0.65a | 4.83 ± 0.67a | 6.37 ± 0.13a | 4.3 ± 0.28a |
| | **Cu (µg/g)** | 35.63 ± 12.29a | 35.57 ± 13.14a | 37.73 ± 14.89a | 22.5 ± 0.78a | 27.8 ± 3.73a | 20.97 ± 0.97a | 27.03 ± 0.87a | 25.17 ± 0.54a |
| | **Pb (µg/g)** | 58.83 ± 3.08a | 59 ± 5.13a | 61.33 ± 4.54a | 11.4 ± 1.34a | 19.33 ± 2.26a | 22.1 ± 0.36a | 26.1 ± 1.34a | 19.5 ± 1.07a |
| | | | | | | | | | |

# SUPLEMENTARY MATERIAL

## TOTAL CONTENTS OF PHENOLICS (TPC) AND FLAVONOIDS (TFC)

**p value TPC**

| | |
|---|---|
| U. rigida vs P. palmata | **~0** |
| U. rigida vs F. vesiculosus | **~0** |
| P. palmata vs F. vesiculosus | **~0** |

**p value TFC**

| | |
|---|---|
| U. rigida vs P. palmata | 0.03 |
| U. rigida vs F. vesiculosus | 0.46 |
| P. palmata vs F. vesiculosus | **0.001** |

## ENZYME

**p-value AChE**

| | |
|---|---|
| Control vs U. rigida cookies | **0.0008** |
| Control vs P. palmata cookies | **0.008** |
| Control vs F. vesiculosus cookies | 0.08 |
| U. rigida cookies vs P. palmata cookies | 1 |
| U. rigida cookies vs F. vesiculosus cookies | 0.46 |
| P. palmata cookies vs F. vesiculosus cookies | 1 |

**p-value BChE**

| | |
|---|---|
| Control vs U. rigida cookies | **0.0007** |
| Control vs P. palmata cookies | **0.02** |
| Control vs F. vesiculosus cookies | 0.08 |
| U. rigida cookies vs P. palmata cookies | 1 |
| U. rigida cookies vs F. vesiculosus cookies | 0.43 |
| P. palmata cookies vs F. vesiculosus cookies | 1 |

## CCA

**p value**

| | |
|---|---|
| Control vs U. rigida cookies | **0.009** |
| Control vs P. palmata cookies | **~0** |
| Control vs F. vesiculosus cookies | 0.43 |
| U. rigida cookies vs P. palmata cookies | 0.43 |
| U. rigida cookies vs F. vesiculosus cookies | |

## FRAP

**p value**

| | |
|---|---|
| Control vs U. rigida cookies | 0.22 |
| Control vs P. palmata cookies | **~0** |
| Control vs F. vesiculosus cookies | 0.03 |
| U. rigida cookies vs P. palmata cookies | 0.03 |
| U. rigida cookies vs F. vesiculosus cookies | 1 |
| P. palmata cookies vs F. vesiculosus cookies | 0.22 |

# SUPLEMENTARY MATERIAL

Calibrated curves:

- bovine serum albumin (BSA), 1 - 0.002mg/mL, in dH2O
- glucose, 1 - 0.002 mg/mL in dH2O)

Method:

- Dpph: sample mixed with 200 μL of DPPH solution (120 μM) in ethanol, and incubated in darkness at RT for 30 min. The absorbance was measured at 517 nm
- ABTS: Sample mixed with 190 μL of ABTS+ solution. After a period of incubation of 6 min in the dark, the absorbance was measured at 734 nm.
- CCA: mixed with 200 μL of 50 mM Na acetate buffer (pH 6), 6 μL of pyrocatechol violet (4 mM) in the above buffer and 100 μL of $CuSO_4$. The absorbance was measured at 632 nm
- FRAP: distilled water (50 μl), and 50 μl 1% (w/v) Potassium hexacyanoferrate were used and incubated at 50 ºC for 20 min. Then, 50 μl of 10 % (w/v) trichloroacetic acid and 10 μl of 0.1 % (w/v) ferric chloride solution were mixed. It was allowed to stand for 10 min at room temperature and absorbance was measured at 700 nm
- TFC: 50 μL of 2% $AlCl_3$-ethanol solution. 50 μL of the extracts or the standard (quercetin) in place of the sample. .Incubate **10 min** at RT. Read the absorbance at 420 nm
- TFC: Mix 5 μL of extracts (10 mg/mL) + 100 μL of F-C reagent (10x diluted)
- TPC: Incubate 10 min at room temperature. Add 100 μL of Sodium Carbonate (75 g/L in dH20). Incubate 90 min at room temperature. Read at 725nm
- Enzyme: 140 μL of sodium phosphate buffer (0.02 mM, pH 8.0), 20 μL of the extract [LMBC1] and 20 μL of AChE or BChE enzymes were mixed in buffer and incubated for 15 min at 25 °C. Then, 20 μL of DTNB (5,5′-dithiobis(2-nitrobenzoic acid) 1.2 mM) and 10 μL of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM) were added for the AChE or BChE assay, respectively. The absorbance was then measured at 412 nm [LMBC1]At what concentrations?