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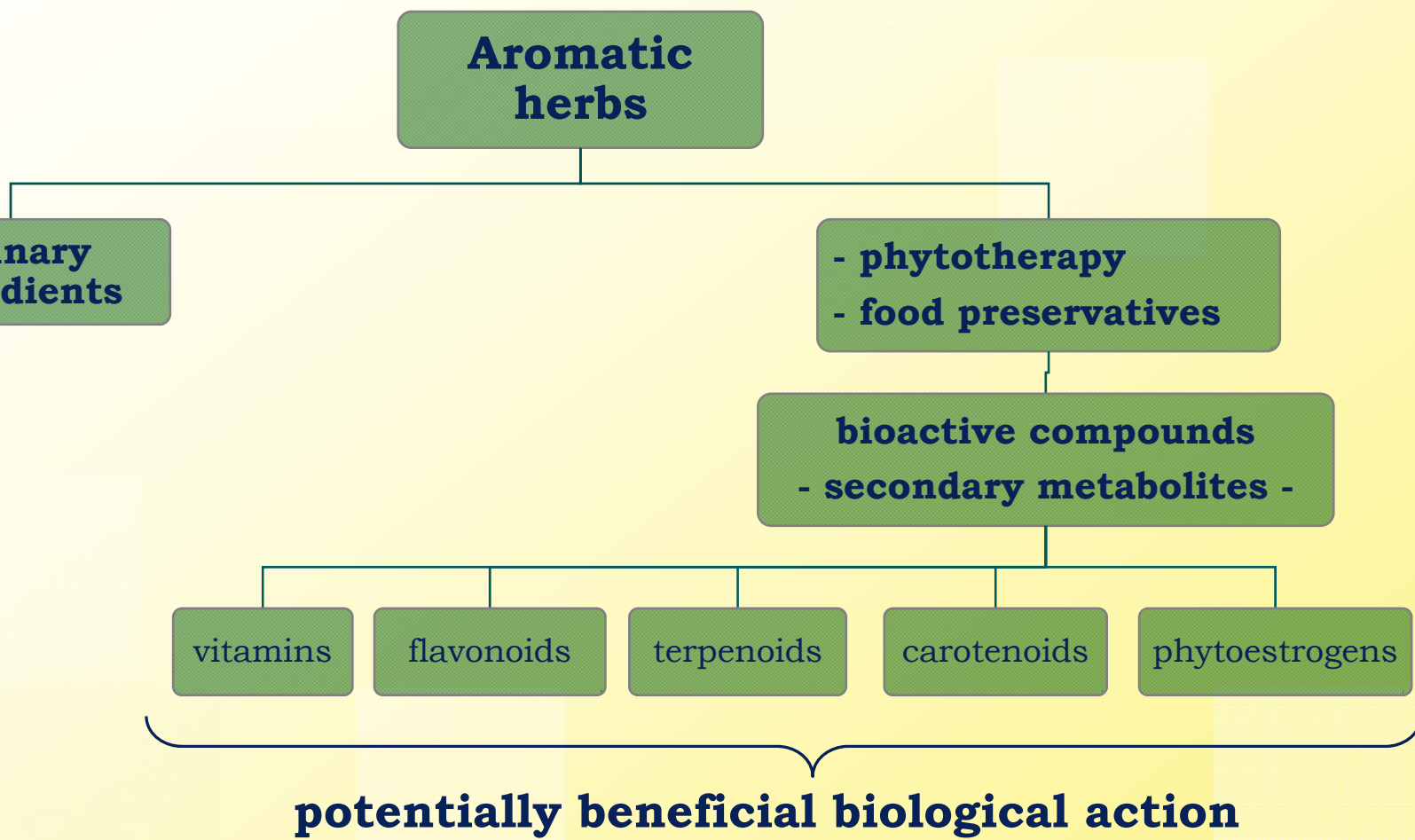
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Effect of Processing Method on the Bioactivity of Parsley (*Petroselinum Crispum* (Mill.) Fuss Var. Neapolitanum)

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- improper handling and storing
- post-harvest mechanical operations

loses of bioactive compounds

decrease of **health benefits** and **preserving properties**

reduces shelf-life

leads to food-related outbreaks



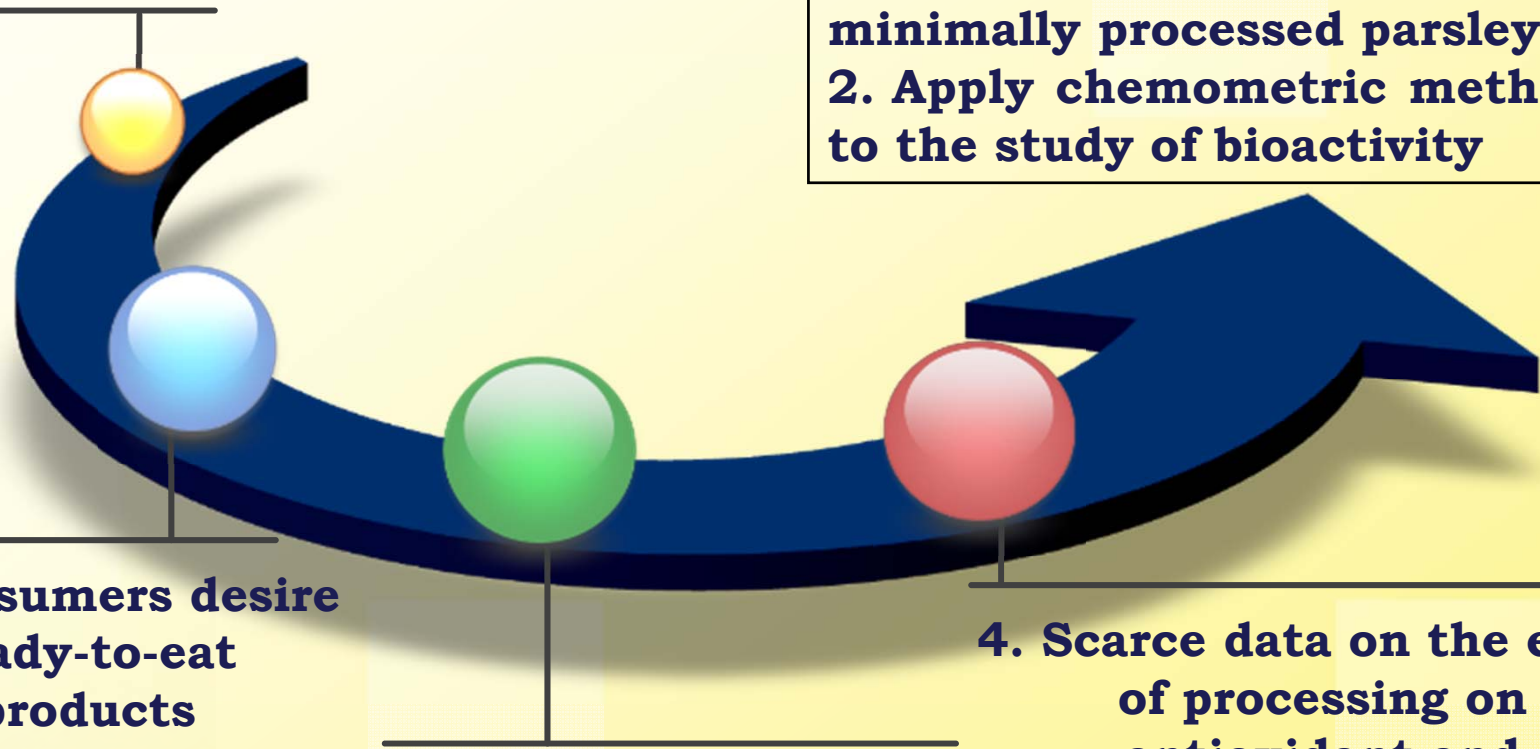
Aims of study

- 1. Determine the bioactivity of minimally processed parsley**
- 2. Apply chemometric methods to the study of bioactivity**

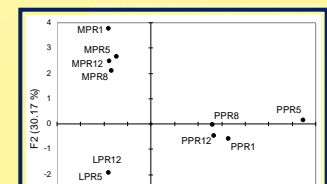
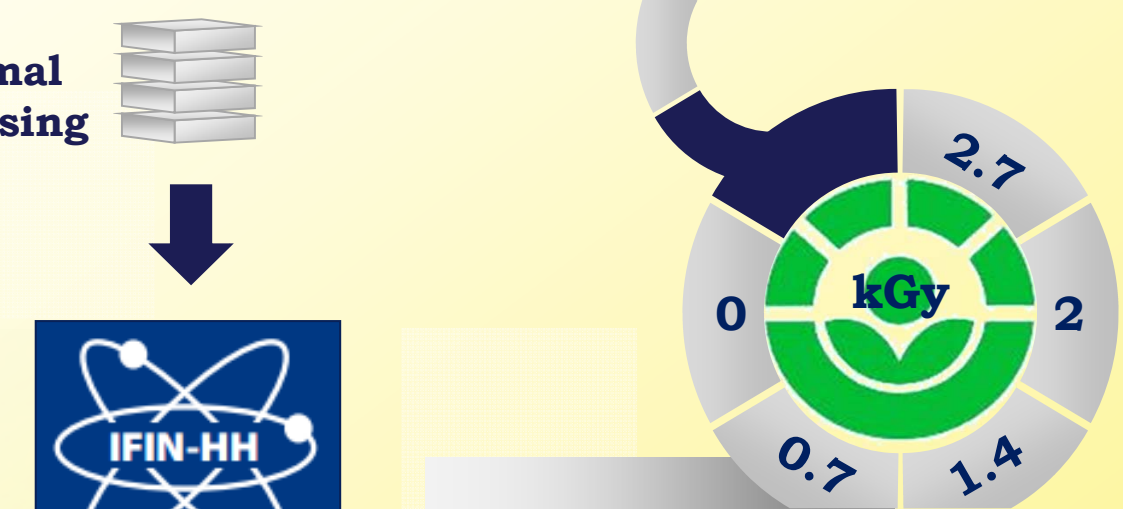
Parsley – rich in bioactive compounds

Consumers desire ready-to-eat products

4. Scarce data on the effect of processing on antioxidant and



Experimental approach



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Methods

Ascorbic acid separation, identification and dosage

HPLC Agilent 1200 system coupled with UV-VIS detector (DAD)

Eclipse XDB-C18 column (5 μm ; 150 x 4.6)

eluted isocratically by a water/acetonitrile/formic acid
(4/5/1; v/v/v) mobile phase

flow rate of 0.5 $\text{ml} \cdot \text{min}^{-1}$

Chromatograms were registered at 240 nm

Total polyphenols

determined spectrophotometrically - Folin-Ciocalteu method

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Antioxidant activity – DPPH radical scavenging method

concentration of **methanolic extracts** of parsley

200, 150, 100, 50, 25 mg FW/ml

positive controls:

vitamin C: 0,30; 0,20; 0,15; 0,10; 0,3 g/l

gallic acid: 0,50; 0,40; 0,30; 0,20; 0,10 g/l

absorbance at **515 nm** (UV-Vis 1700 PharmaSpec Shimadzu)

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Antioxidant activity – DPPH radical scavenging method

Indexes were computed:

antioxidant activity (**I%**)

50% free radical scavenging activity (**IC₅₀**)

radical scavenging activity (**ARA**)

ascorbic acid equivalent antioxidant capacity and antioxidant activity index (**AAI**)

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Antibacterial activity – Kirby-Bauer disc diffusion assay

concentrations of **methanolic extracts** of parsley:

200, 150, 100, 50, 25 mg FW/ml

positive control **gentamicin sulphate 40 mg/ml**

negative control **methanol**

Bacillus cereus – ATCC 11778

Stafilococcus auerus – ATCC 49444

Escherichia coli – ATCC 25922

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Antibacterial activity – Kirby-Bauer disc diffusion assay

Indexes were computed:

the diameter of inhibition zone (**DIZ**)

a **Fisher correlation** between gentamicin concentration and DIZ using gentamicine antibiogram for each microorganism

gentamicin equivalent antimicrobial activity (**GEEA**) using inhibition curves

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Methods

Statistics - **XLSTAT** software

- means
- standard deviations
- ANOVA (p -value < 0.05)
- Fisher (LSD, p -value < 0.05)
- Pearson coefficient
- Principal component analysis



Table 1. The content of ascorbic acid and phenols of minimally processed, dried and frozen parsley

Treatment	Content, as mean \pm S.D	
	ascorbic acid (mg \cdot 0.01 g ⁻¹ FW)	total polyphenols (mg GAE \cdot 0.01 g ⁻¹ FW)
Control	192.68 \pm 1.40 a	230.81 \pm 7.82 b
0.7	146.00 \pm 3.61 c	256.45 \pm 10.91 a
Irradiation, (kGy)	1.4	139.76 \pm 2.80 d
	2.0	136.80 \pm 2.21 de
	2.7	134.13 \pm 3.44 e
Drying	57.41 \pm 3.36 f	118.98 \pm 2.00 e
Freezing	182.36 \pm 2.09 b	253.79 \pm 6.85 a

Note: Different letters among treatments denote significant differences (LSD test, $p < 0.05$)

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Table 2. Antioxidant activity indexes of minimally processed, dried and frozen parsley

Treatment	Content, as mean ± S.D.				
	IC ₅₀ (µg · ml ⁻¹)	ARA (mg DPPH · 0.01 g ⁻¹ FW)	AEAC (mg vitamin C · 0.01 g ⁻¹ FW)	AAI · 10 ⁻³	
Control	450.0 ± 0.1 d	6.5 ± 0.08 c	131.2 ± 0.1 c	51.8 ± 0.0 b	
0.7	494.6 ± 0.6 cd	5.5 ± 0.1 c	119.3 ± 0.1 c	47.1 ± 0.1 b	
radiation, (Gy)	1.4	513.3 ± 18.3 cd	5.9 ± 0.2 c	114.9 ± 4.1 c	45.4 ± 1.6 b
2.0	470.3 ± 0.0 d	5.1 ± 0.2 c	125.1 ± 0.2 c	37.9 ± 0.0 b	
2.7	753.8 ± 283.5 c	3.8 ± 0.1 c	107.0 ± 0.9 c	25.5 ± 9.6 b	
drying	6,892.6 ± 240.5 a	0.5 ± 0.1 c	8.6 ± 0.7 d	3.9 ± 0.1 b	
freezing	1,811.3 ± 140.3 b	1.7 ± 0.3 c	32.5 ± 2.4 d	14.7 ± 1.1b	
Ascorbic acid	0.8 ± 0.1 e	3,822.0 ± 5.4 a	71,746.0 ± 97.8 b	30,595.9 ± 40.2 a	
Vitamin C	0.6 ± 0.1 e	3,745.8 ± 10.8 b	100,000.0 a	30,042.2 ± 67.8 a	

Note: Different letters among treatments denote significant

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Table 3. Diameter of inhibition zone (DIZ) of minimally processed, frozen and dried parsley against *Salmonella Typhimurium*

Treatment	Extract concentration, mean ± S.D. (mg FW · ml ⁻¹)				
	200	150	100	50	25
Control	24.75 ± 0.35 b	16.50 ± 0.71 b	11.75 ± 0.35 c	9.25 ± 0.35 c	8.00 ± 0.71 c
0.7	28.50 ± 0.71 a	17.50 ± 0.71 b	11.75 ± 0.35 c	10.00 ± 0.71 c	8.25 ± 0.35 b
1.4	20.50 ± 0.71 cd	15.00 ± 0.71 c	10.00 ± 0.00 d	6.75 ± 0.35 d	6.00 c
2.0	19.50 ± 0.71 de	19.00 ± 0.00 a	15.50 ± 0.71 b	12.75 ± 1.06 b	11.00 ± 0.00 a
2.7	21.00 ± 0.71 c	19.00 ± 0.71 a	17.50 ± 0.71 a	15.00 ± 0.71 a	8.50 ± 0.71 b
drying	19.00 ± 0.71 e	13.50 ± 0.71 d	10.00 ± 0.00 d	7.25 ± 0.35 d	6.50 ± 0.00 c
freezing	6.00 f	6.00 e	6.00 e	6.00 e	6.00 e

Note: Different letters among treatments denote significant differences among treatments (LSD test, p < 0.05).

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Table 4. Diameter of inhibition zone (DIZ) of minimally processed, frozen and dried parsley against *Bacillus cereus*

Treatment	Extract concentration, mean ± S.D. (mg FW · ml ⁻¹)				
	200	150	100	50	25
Control	16.25 ± 0.35 a	14.50 ± 0.71 b	11.75 ± 0.35 ab	7.25 ± 0.35 c	6.00
0.7	16.75 ± 0.35 a	15.25 ± 0.35 a	11.50 ± 0.71 ab	8.75 ± 0.35 a	6.00
1.4	13.50 ± 0.71 b	12.50 ± 0.00 c	10.00 ± 0.71 b	6.00 d	6.00
2.0	15.50 ± 0.71 a	14.75 ± 0.35 ab	10.75 ± 0.35 ab	8.00 ± 0.00 b	6.00
2.7	16.50 ± 0.00 a	15.00 ± 0.00 a	12.25 ± 0.35 ab	8.50 ± 0.00 a	6.00
Freezing	8.00 ± 0.00	7.50 ± 0.00	6.00	6.00	6.00
Drying	6.00	6.00	6.00	6.00	6.00

Note: Different letters among treatments denote significant differences among treatments (LSD test, $p < 0.05$).

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Table 5. Diameter of inhibition zone (DIZ) of minimally processed, frozen and dried parsley against *Staphylococcus aureus*

Treatment	Extract concentration, (mg FW · ml ⁻¹)				
	200	150	100	50	25
Control	12.50 ± 0.71 a	10.50 ± 0.71 b	6.75 ± 0.35 b	6.00 a	6.00
0.7	10.00 ± 0.00 b	7.25 ± 0.35 c	6.00 c	6.00 a	6.00
1.4	9.50 ± 0.71 b	7.75 ± 0.35 c	6.25 ± 0.35 bc	6.00 a	6.00
2.0 (Gy)	13.00 ± 0.00 a	11.75 ± 0.35 a	7.75 ± 0.35 a	6.25 ± 0.35 a	6.00
2.7	12.75 ± 0.35	9.50 ± 0.71	7.75 ± 0.35	6.00 a	6.00
Drying	6.00 c	6.00 d	6.00c	6.00 a	6.00
Freezing	6.00 c	6.0 d	6.00 c	6.00 a	6.00

Note: Different letters among treatments denote significant differences among treatments (LSD test, p < 0.05).

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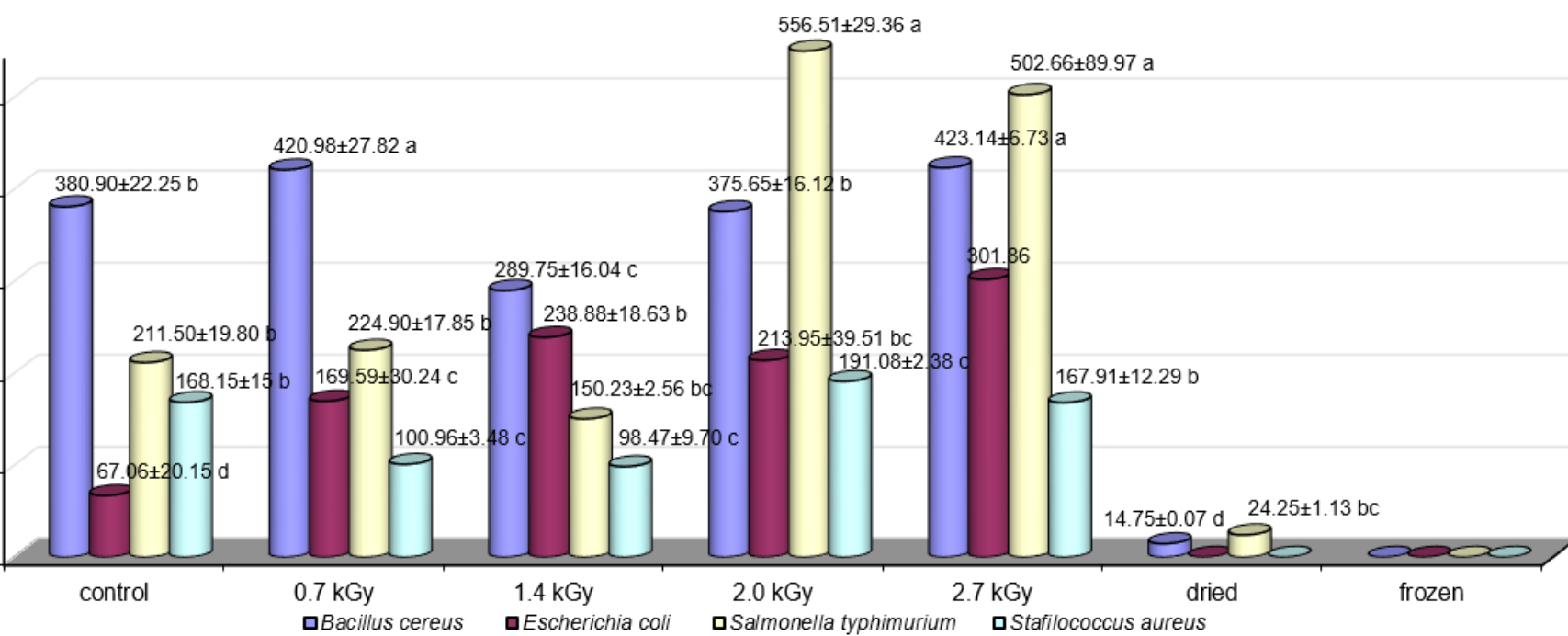
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Table 6. Diameter of inhibition zone (DIZ) of minimally processed, frozen and dried parsley against *Escherichia coli*

Treatment	Extract concentration, mean ± S.D. (mg FW · ml ⁻¹)				
	200	150	100	50	25
Control	7.25 ± 0.35 c	6.75 ± 0.35 c	6.00 b	6.00 c	6.00
0.7	9.50 ± 0.71 b	8.50 ± 0.71 b	6.00 b	6.00 c	6.00
1.4	12.00 ± 0.71 a	8.75 ± 0.35 b	6.75 ± 1.06 ab	6.25 ± 0.35 bc	6.00
2.0	10.50 ± 0.71 b	9.50 ± 0.71 b	7.25 ± 0.35 a	6.75 ± 0.35 a	6.50 ± 0.71
2.7	13.00 ± 0.71 a	11.50 ± 0.71 a	7.25 ± 0.35 a	6.50 ± 0.00 ab	6.00
drying	6.00 c	6.00 c	6.00 b	6.00 c	6.00
freezing	6.25 ± 0.35 c	6.00 c	6.00 b	6.00 c	6.00

Note: Different letters among treatments denote significant differences among treatments (LSD test, $p < 0.05$).

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: Different letters stand for significant statistical differences for each microorganism (Fisher (LSD), p < 0.5).

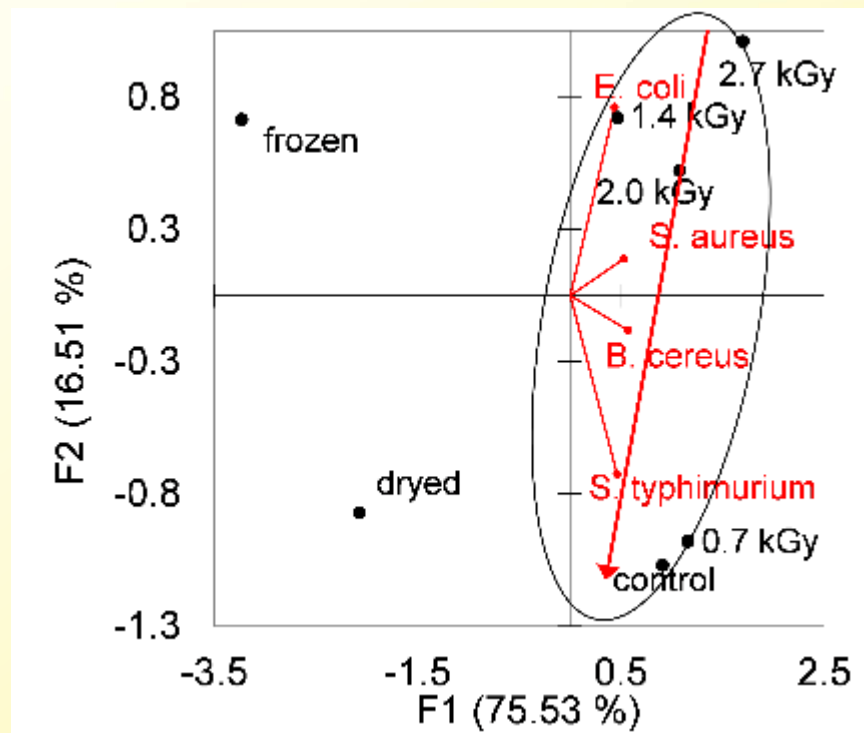


Figure 2. Principal component analysis (PCA) of antimicrobial

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Irradiation decreased the content of vitamin C by 30% for a 2.0 kGy dose, but increased total polyphenols in samples irradiated with doses below 2.0 kGy.

Drying caused a significant loss of vitamin C and phenols.

Minimally processed parsley showed a significant antibacterial activity. Resistance to parsley extracts was: *E. coli* > *S. aureus* > *S. Typhimurium*.

Irradiation increased the susceptibility of Gram-negative bacteria to parsley extracts.

Freezing and drying affected the antioxidant and antimicrobial activity of parsley to a greater extent than ionizing radiation treatment; irradiated samples exhibited bioactivity similar to fresh parsley.

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Absorbed doses of 0.7-1.4 kGy can be recommended to no ment because the content of vitamin C was better preserved and nificant increase of polyphenols was observed when compared control samples.

Drying can be recommended only in terms of vitamin C and henols content per consumed serving, as it doubles the content amin C and triples polyphenols compared to the same mass of product.

Plant extracts with optimal antioxidant and antimicrobial can obtained from fresh herbs stored at refrigeration temperature as as possible.

An absorbed dose of 2.7 kGy may be applied to parsley in order crease its antibacterial against Gram-negative bacteria such as

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