







NPA

Natural products application

Health, Cosmetic and Food

Online Edition

4-5 Feb 2021

## Introduction

- > Pumpkin, (Cucurbita pepo L.) is one of the most important vegetables of the Cucurbitaceae family which is widely used for its edible fleshy fruit, especially fruit pulp.
- Cucurbitaceae family, includes 130 genera and more than 800 species (Perez-Gutierrez, 2016). The different species offer a diversity of fruit characteristics such as shape, size, color, taste, and seeds (Gong et al., 2012).
- The popularity of the pumpkin for use in various traditional medicines for several ailments (antidiabetic, antihypertensive, antitumour, immunomodulation, antibacterial, antalgic, antihypercholesterolemia, intestinal antiparasitia, anti-inflammation) has attracted scientific attention to this plant (Fu et al., 2006).
- > Pumpkin oil press-cake has a substantial amount of residual oil, which is rich in omega-6 fatty acids, tocopherols, minerals and proteins, and as such, could have different applications in the development of functional food products (Radočaj et al., 2011a).
- > In the present work, the chemical composition and bioactive properties of pumpkin seeds and seed cakes were evaluated.

# Materials and Methods

- > Pumpkin seeds (*Cucurbita pepo* L.) of the local landrace "Nychaki" were sown directly in soil on 27/7/2020 at the University of Thessaly during the summer-autumn growing period of 2020.
- $\blacktriangleright$  Plant distances were 2.5 m between rows and 0.80 m within rows (4705 plants/ha).
- Fruit were collected at marketable maturity on 20/11/2020 and seeds were removed from 15 randomly selected after cutting each fruit at the equatorial axis.
- > The seeds were air-dried at room temperature and pressed with a cold-press to obtain the seed cakes, while whole air-dried seeds were ground to fine powder.
- Nutritional value was assessed according to AOAC (2016).
- > Tocopherols, free sugars and organic acids were analyzed with high performance liquid chromatography (HPLC).
- > Fatty acids obtained with Soxhlet apparatus were analyzed by gas liquid chromatography after transesterification of the lipid fraction.
- > Cytotoxicity was determined on a non-tumour primary culture of porcine liver cells (PLP2 cells).
- > For chemical composition analyses three batch samples from the collected seeds were used (n=3). Data were evaluated by a one-way ANOVA, while the means of values were compared with Tukey's HSD test (p=0.05).



**Image 1.** Pumpkins (*Cucurbita pepo* L.)



Image 2. Pumpkin seeds

Table 3. Composition in sugar (g/100 g dw) of the studied cucurbit ground seeds and seedcake (mean  $\pm$ 



Positive control (Ellipticine).  $GI_{50}$  values (3.2±0.7 µg/mL), corresponds to the sample concentration achieving 50% in liver primary culture PLP2.

# **CHEMICAL COMPOSITION AND BIOACTIVE PROPERTIES OF PUMPKIN SEEDS AND SEED CAKES**

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# Results

**Table 1.** Nutritional value (g/100 g dw) and energetic value (kcal/100 g dw) of the studied cucurbit ground seeds and seedcake (mean  $\pm$  SD).

	Fat	Proteins	Ash	Carbohydrates	Energy
ound seeds	42.74±0.09	37.7±0.2	3.52±0.09	16.07±0.03	599.6±0.1
seedcake	7.62±0.08	58.6±0.3	5.40±0.05	28.4±0.2	416.5±0.4

**Table 2.** Composition in tocopherols (mg/100 g dw) of the studied cucurbit ground seeds and seedcake (mean  $\pm$  SD).

	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total Tocopherols
ound seeds	0.075±0.004	0.011±0.001	6.59±0.03	0.28±0.01	6.96±0.02
seedcake	0.018±0.001	0.079±0.002	1.07±0.04	0.016±0.002	1.18±0.04

	Fructose	Glucose	Sucrose	Trehalose	Total Sugars
ound seeds	0.20±0.01	0.21±0.01	1.97±0.04	0.26±0.01	2.6±0.1
seedcake	0.34±0.01	0.19±0.01	2.9±0.1	0.25±0.01	3.7±0.1

**Table 4.** Composition in organic acids (g/100 g dw) of the studied cucurbit ground seeds and seedcake (mean  $\pm$  SD).

	Oxalic acid	Malic acid	Total organic acids
curbit seeds ground	tr	tr	_
Cucurbit cake	0.006±0.001	tr	0.006±0.001

tr- traces

**Table 5.** Cytotoxicity of the studied cucurbit ground seeds and seedcake ( $GI_{50}$  values  $\mu g/mL$ ).

	Hepatotoxicity PLP2 (non-tumor cells)
Cucurbit seeds ground	>400
Cucurbit cake	>400



<b>le 6.</b> Fatty acids composition (%) of the studied cucurbit ground seeds and seedcake (mean $\pm$ SD).			
	Cucurbit seeds ground	Cucurbit cake	
C6:0	0.015±0.001	0.168±0.006	
C8:0	0.002±0.001	0.022±0.001	
C10:0	0.006±0.001	0.011±0.001	
C12:0	0.020±0.001	0.039±0.001	
C14:0	0.117±0.004	0.230±0.006	
C15:0	0.020±0.001	0.043±0.001	
C16:0	12.20±0.04	14.0±0.4	
C16:1	0.119±0.004	0.162±0.001	
C17:0	0.094±0.004	0.092±0.003	
C18:0	4.83±0.08	5.46±0.02	
C18:1n9c+t	37.0±0.1	36.27±0.02	
C18:2n6c	43.89±0.01	41.5±0.3	
C18:3n3	0.242±0.004	0.585±0.001	
C20:0	0.359±0.004	0.400±0.002	
C20:1	0.192±0.001	0.242±0.005	
C20:3n3+C21:0	0.27±0.01	0.154±0.005	
C20:5n3	0.11±0.01	0.063±0.001	
C22:0	0.294±0.009	0.43±0.02	
C22:1n9	0.048±0.003	0.016±0.001	
C23:0	0.027±0.001	0.064±0.001	
C24:0	0.118±0.003	0.062±0.001	
Total SFA (% of total FA)	18.10±0.03	21.0±0.3	
Total MUFA (% of total FA)	37.4±0.1	36.69±0.01	
Total PUFA (% of total FA)	44.51±0.09	42.3±0.3	

> The ground seeds were rich in fat and proteins while seed cakes contained a high amount of protein and carbohydrates.

 $\blacktriangleright$  Ground seeds and seed cakes contained all the four vitamin E isoforms with y-tocopherol being the most abundant isomer in both samples.

> The main detected free sugar in ground seeds and seed cakes were sucrose, followed by trehalose, fructose and glucose, while seed cakes contained a higher amount of sucrose and total free sugars than ground seeds.

> Oxalic acid content was the only detected compound in seed cakes, whereas no organic acids were detected in ground seeds.

> The main detected fatty acids were linoleic acid (43.9% and 41.5% in ground seeds and seed cakes, respectively) and oleic acid (37.0% and 36.3% in ground seeds and seed cakes, respectively), followed by stearic acid (4.83% and 5.46% in ground seeds and seed cakes, respectively). Polyunsaturated and monounsaturated fatty acids were the main fatty acids class and accounted for 81.9% and 79.0% of total fatty acids in ground seeds and seed cakes, respectively.

The project is funded by the General Secretariat for Research and Technology of the Ministry of Development and Investments under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union's Programme for Research and Innovation (Prima2019-08). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020); for the financial support through national funding from the FCT, within the scope of the Project PRIMA Section 2 - Multi-topic 2019: Pulping (PRIMA/0007/2019); and L. Barros and Â. Fernandes thank the national funding by FCT, P.I., through the institutional scientific employment program-contract for their contacts.

### Conclusion

Both pumpkin seeds and seed cakes showed no toxic effects against nontumor PLP2 cell lines indicating that they are safe for human consumption.

he presented results highlighted the nutritional value of the pumpkin seeds and seed cakes which could be considered a rich source of protein.

he high content in polyunsaturated fatty acids and tocopherols due to results could be further valorized for pharmaceutical and nutraceutical purposes and ncrease the added value of pumpkin crop.

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### Acknowledgements