CRYOGENIC GRINDING IN EVALUATION OF BIOACTIVE POTENTIAL OF PUMPKIN SEED CAKE

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INTRODUCTION

The food industry is constantly confronted with a large amount of waste that needs to be properly disposed. An important option in reducing the amount of waste is to utilize these by-products. Oil pumpkin (*Cucurbita pepo* L., var. *Styriaca*) is certainly one of the cultivars with the great potential of waste and by-product exploitation primarily because of the high amount of waste that results from the production of pumpkin seed oil. So far, cryogenic grinding is limited to the production of functional products and dietary supplements in which the added value of the product is sufficient to cover for the expense. However, since some of the compounds potentially present in pumpkin seed cake, such as chlorophylls and phytosterols, have excellent potential in the dietary supplement market because of their specific nutritional and medical effects, the impact of cryogenic grinding on the increase of these compounds has been investigated. The specific aim of this work was to assess the effect of cryogenic conditions application as well as grinding time, as the independent variables, on the yield of the bioactive molecules and the antioxidant activity of the pumpkin seed cake non-polar and polar extracts.

MILLING TREATMENT
Without cooling (WC)
Cryogenic cooling (C)
Cryogenic cooling with intermediary pauses (CI)

MILLING TIME
4, 8, 12 minutes

Hexane extraction → **NON-POLAR EXTRACT**
Fatty acids, squalene, phytosterols, tocopherols

Acetone/water extraction → **POLAR EXTRACT**
Phenols, protochlorophyll, antioxidant activity

MATERIALS AND METHODS

Pumpkin seed cake formed by hydraulic pressing was grinded in a laboratory mill and sifted through a 1 mm diameter sift to obtain a starting (control) sample. The control sample was milled on "CryoMill" (Retsch, Germany) based on ball milling according to the experimental design shown in table 1. Samples were processed for 4, 8 and 12 minutes through 3 treatments: without liquid nitrogen cooling, with liquid nitrogen cooling and with intermediary liquid nitrogen cooling during which the mill oscillations were paused for a period of 2 minutes after every 2 minutes of milling. Non-polar and polar extracts were prepared by applying hexane followed by acetone/water mixture (80:20, v/v). The protochlorophyll content was determined spectrophotometrically according to the method developed by Anderson and Boardman (1964). The total phenols were determined according to Čukelj et al (2015). Antioxidant activity was determined by DPPH method according to Čukelj et al. (2015). Fatty acid methyl-esters (FAME) were prepared according to the standard ISO method (HRN EN ISO 5009: 2004). Sterols, tocopherol and squalene were determined by modification of HR EN ISO 12228 (2014).

RESULTS

Table 1. Extraction yields (%, average ± standard deviation, n=3)

Exp.	Hexane extract yield†,ǂ (%)	Acetone/water extract yield‡ (%)
Control	11,83 ± 0,02	6,83 ± 0,32
4WC	11,76 ± 0,06	8,10 ± 0,05
8WC	11,85 ± 0,14	7,88 ± 0,04
12WC	12,27 ± 0,16	8,23 ± 0,10
4C	11,02 ± 0,04	8,59 ± 0,19
8C	11,78 ± 0,02	8,82 ± 0,18
12C	12,64 ± 0,08	8,76 ±0,22
4CI	12,38 ± 0,12	7,84 ± 0,48
8CI	12,55 ± 0,24	8,16 ±0,11
12CI	12,76 ± 0,04	8,06 ± 0,19

† Significant influence of time ($p \leq 0,05$)
ǂ Significant influence of treatment ($p \leq 0,05$)

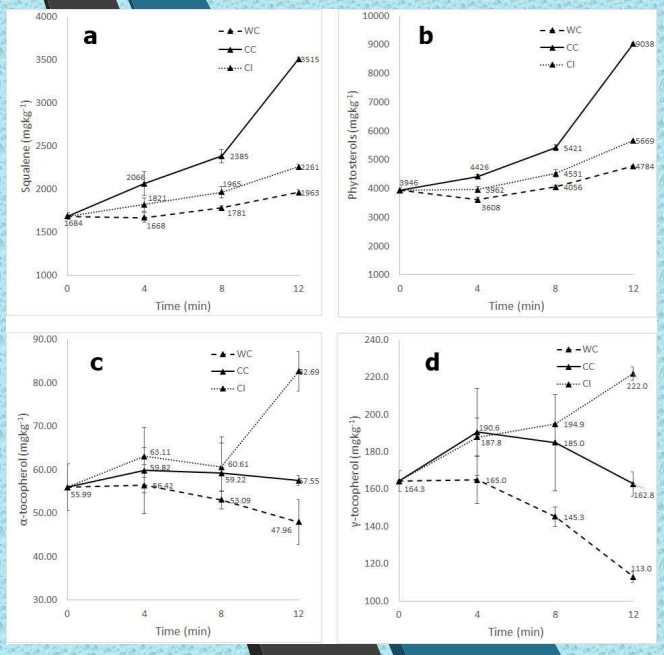

Figure 1. Squalene (a), total sterols (b), γ-tocopherol (c) and α-tocopherol (d) content* (average ± standard deviation, n=3) in hexane extracts

Table 2. Protochlorophylls, total phenols (TPC) and antioxidant activity * (%, average ± standard deviation, n=3) in acetone/water extracts

Exp.	Protochlorophyll †,ǂ,∫ (mg kg-1)	TPC †,ǂ,∫ (mg 100 g-1)	Antioxidant activity†,ǂ,∫ (mM TE g-1)
Control	72.6±0.6^{a}	9.0±0.2^{a}	0.99±0.05^{a}
4WC	84.8±6.8^{a}	10.6±0.2^{b,c}	1.20±0.03^{b}
8WC	144.9±2.3^{b,c}	10.0±0.7^{a,b}	1.21±0.07^{b,c}
12WC	155.2±2.1^{c,d}	10.5±0.4^{b,c}	1.20±0.05^{b}
4C	146.9±2.2^{b,c}	11.9±0.2^{c,d}	1.33±0.05^{b,c,d}
8C	172.4±4.7^{d,e,f}	11.9±0.1^{c,d}	1.42±0.00^{d,e}
12C	169.5±11.2^{d,e}	12.3±0.4^{d}	1.79±0.02^{f}
4CI	131.2±7.0^{b}	11.1±0.4^{b,c,d}	1.20±0.02^{b}
8CI	185.7±1.1^{e,f}	11.0±0.5^{b,c,d}	1.37±0.02^{c,d}
12CI	188.8±1.3^{f}	12.2±0.4^{d}	1.56±0.01^{f}

† Significant influence of time ($p \leq 0,05$)
ǂ Significant influence of treatment ($p \leq 0,05$)
∫ Significant influence of time x treatment interaction ($p \leq 0,05$)
* Means with the same letter are not significantly different ($p \leq 0,05$)

Table 3. Fatty acid profile* (%, average ± standard deviation, n=3) in hexane extracts

Exp.	C16:0†	C18:0	C18:1†,∫	C18:2	C18:3†	C20:0
Control	11.5±0.1^{a}	6.0±0.0^{a}	42.8±0.1^{a}	38.4±0.1^{a}	0.2±0.1^{a}	0.4±0.0^{a}
4WC	11.5±0.0^{a,b}	6.0±0.0^{a}	42.7±0.0^{a,b}	38.3±0.1^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
8WC	11.5±0.0^{a,b}	6.0±0.0^{a}	42.6±0.1^{b}	38.3±0.1^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
12WC	11.5±0.0^{a,b}	6.0±0.0^{a}	42.7±0.1^{a,b}	38.3±0.1^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
4C	11.5±0.0^{a,b}	6.0±0.0^{a}	42.7±0.0^{a,b}	38.3±0.1^{a}	0.3±0.0^{a,b}	0.4±0.1^{a}
8C	11.5±0.1^{a,b}	6.0±0.0^{a}	42.7±0.0^{a,b}	38.4±0.1^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
12C	11.5±0.0^{a,b}	6.0±0.0^{a}	42.7±0.0^{a,b}	38.4±0.0^{a}	0.4±0.1^{b,c}	0.4±0.0^{a}
4CI	11.6±0.1^{a,b}	6.0±0.0^{a}	42.6±0.1^{b}	38.4±0.1^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
8CI	11.5±0.0^{a,b}	6.0±0.0^{a}	42.7±0.0^{a,b}	38.4±0.0^{a}	0.3±0.0^{a,b}	0.4±0.0^{a}
12CI	11.6±0.1^{b}	6.0±0.0^{a}	42.6±0.1^{b}	38.4±0.0^{a}	0.4±0.0^{c}	0.4±0.0^{a}

† Significant influence of time ($p \leq 0,05$)
ǂ Significant influence of treatment ($p \leq 0,05$)
∫ Significant influence of time x treatment interaction ($p \leq 0,05$)
* Means with the same letter are not significantly different ($p \leq 0,05$)

CONCLUSIONS

1. The analyses showed an exceptional nutritional value of pumpkin seed cake, i.e. that it contains high levels of protochlorophyll, phenol, squalene, tocopherol and phytosterol that have an important influence on physiological function and human health.
2. Statistical analysis of the results showed a significant influence of the cryogenic grinding on the yield of the analyzed bioactive molecules.
3. The samples grinded with freezing had up to 2.5 times larger amounts of target bioactive molecules, especially chlorophylls and phytosterols.
4. Pretreatment of cryogenic grinding is recommended in the extraction of pumpkin seed cake in the production of functional food and dietary supplements.