Study and characterization of novel ingredients recovered from plant material for application in novel foods, by advanced chromatographic technique and high-resolution mass spectrometry

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1 INTRODUCTION *AND AIM*

The hemp fiber supply chain creates several by-products such as seeds. Hemp seeds are an interesting source of proteins, dietary fiber, high-quality fat and phytochemicals, so they might be included in foodstuffs in the form of flour (Rusu et al. 2021). Furthermore, hemp seed can be sprouted to develop microgreens seedlings that can be consumed as unaltered state or added as ingredients in food preparations, thanks to their good phytochemical profile. To investigate this latter a NADES-assisted and conventional extraction were used. NADES are used as solvents for the recovery of bioactive compound from various sources, such as agri-food residues and plant materials (González-Laredo et al., 2023).

The aim of this work was to investigate different ways of using hemp seeds from hemp fiber supply chain, in the form of flour to fortify bread and as source to produce microgreens.

2 METHODS

Hempseed bread:Lipid and protein were determined using standard procedures (AOAC, 2006). Fatty acid profile was obtained by means of GC-MS technique as reported Annaratone et al. (2009). Bound amino acids were determined according to Caligiani et al. (2007). The volatile fraction was performed according to the protocol proposed by Cirlini et al. (2012), based on HS-SPME/GC-MS approach.

Hemp microgreens and Nades preparation:

Three different NADES mainly consisted of L-proline and Glycerol as hydrogen bond acceptors and different hydrogen bond donors such as citric and acetic acid were used and produced by Dai et al. (2013). NADES extraction was performed according Popovic et al. (2012) while conventional extraction by Dall'Asta et al. (2013). Screening assays were conducted follow protocol proposed by Martin-Diana et al. (2017) and Keskin et al. (2019). Target and suspect analysis was performed according Torrijos et al 2022.

4 CONCLUSIONS

Hempseed bread:

the use of hemp seeds as fortifying ingredient increses several chemical parameters (protein, lipid such as fatty acid belonging to omega 3 series, amino acids, TPC and antioxidant activity). This study shows that adding hemp seed flour to bread enhances its nutritionl properties, especially when hemp flour represent hight percetage, and the fortification does not significantly affect the odor and flavor compared to control bread. Thus, adding hemp seed flour as a nutritional element to the recipe for fortified bread adds value by expanding the food range and creating new, nutritious products.

Hemp microgreens:

Our study provides basic information on the richness of bioactive compounds in hemp microgreen extracts from two varieties (Fedora and Futura) using different extraction methods, highlighting their potential as new ingredients. The composition of bioactive compounds was found to be affected by the extraction methods. The use of NADES showed better extractive capacity for phytochemicals compared to ethanol.

5 ACKNOWLEDGMENT

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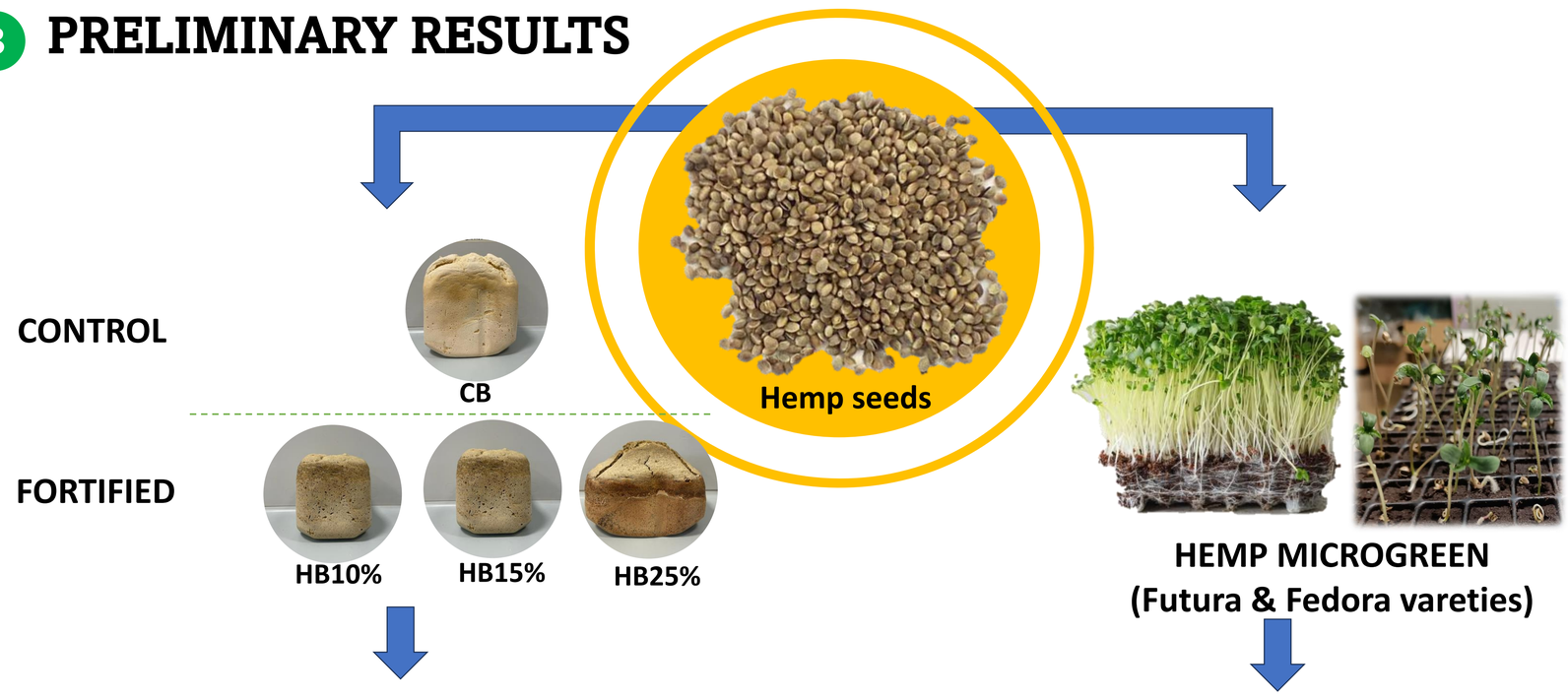
3 PRELIMINARY RESULTS

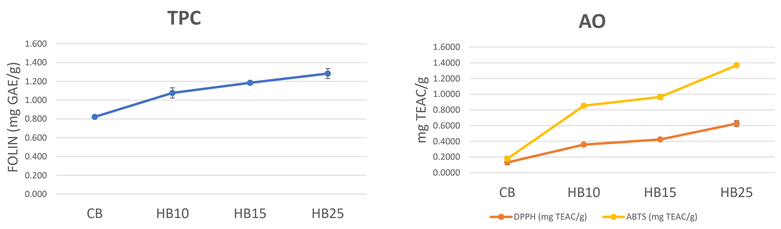


Average composition of proteins, lipids and dry matter of bread fortified with hempseed flour (%)

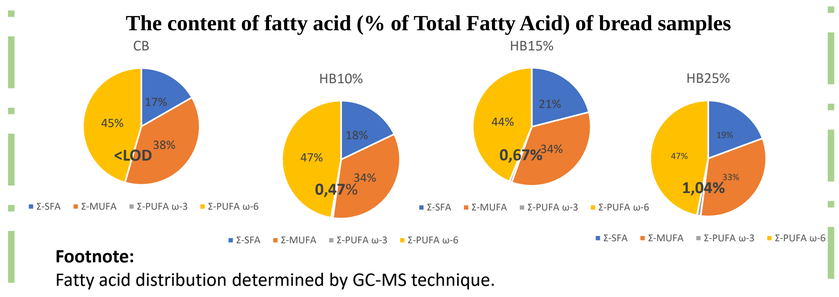
Bread samples	Lipids	Proteins	Dry matter
CB	3,05 ± 0,14$_d$	10,62 ± 0,09$_c$	67,03 ± 0,61$_a$
HB10	4,03 ± 0,02$_c$	12,97 ± 0,06$_b$	68,54 ± 2,82$_a$
HB15	4,51 ± 0,17$_b$	13,21 ± 0,23$_b$	66,93 ± 0,31$_a$
HB25	5,77 ± 0,18$_a$	14,98 ± 0,29$_a$	66,68 ± 1,60$_a$

Footnote:
CB refers to control bread, while HB10-15-25% refers to bread with different percentage of fortification. Protein and lipid content were determined by AOAC methods. Different letters mean a statistical difference (p< 0,05).

Screening assays for Total Polyphenolic content (TPC) and Antioxidant Activity (AO) of bread samples enriched with hempseed flour



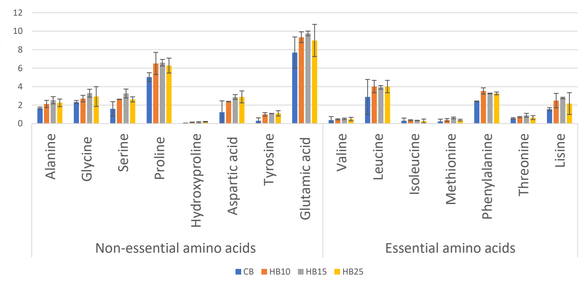
The content of fatty acid (% of Total Fatty Acid) of bread samples



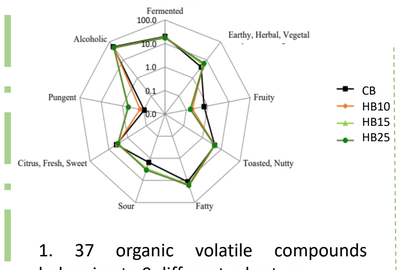
Footnote:
Fatty acid distribution determined by GC-MS technique.

Total amino acid (mg/g sample) in hempseed bread

1. Amino acid profile is rich in essential amino acids;
2. Serine, proline, hydroxyproline, aspartic acid, phenylanine and tyrosine are more abundant in fortified bread.

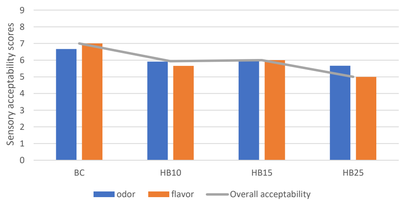


Volatile Organic Compounds (main odor types) and Sensory acceptability

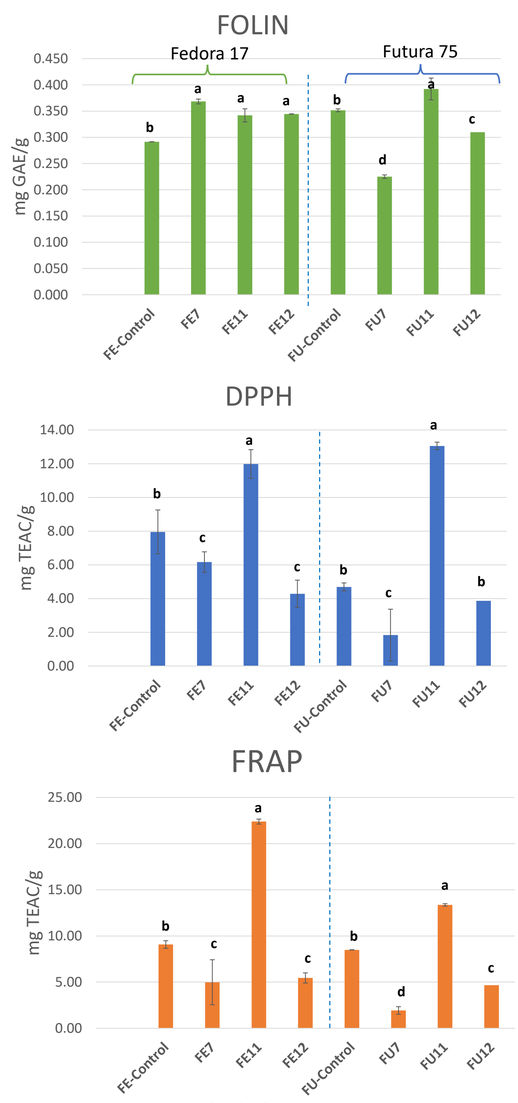


1. 37 organic volatile compounds belonging to 9 different odor type;
2. The main representative are 1-pentanol (pungent), 2-nonanle and 2-octanal (herbal), citral (citrus).

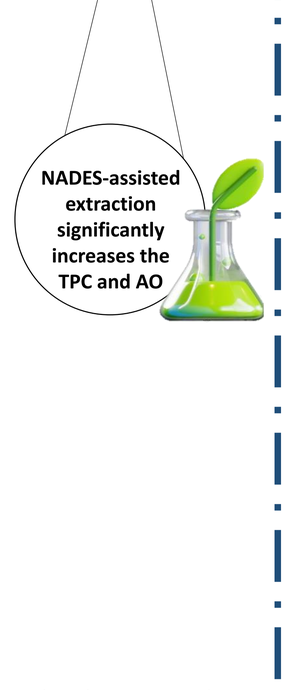
1. Odor and flavor of the fortified bread is not significantly different from the control bread;
2. The overall acceptability decreases when hempseed flour was increased in bread formulation



Screning assays: Total Polyphenolic content (TPC) and Antioxidant Activity (AO)



NADES7	L-proline	Citric a.
NADES11	L-proline	Acetic a.
NADES12	Glycerol	Citric a.

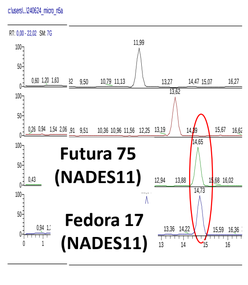


The spectrophotometric results for the following samples were obtained: FE7 and FU7 (NADES7 extracts), FE11 and FU11 (NADES11 extracts), and FE12 and FU12 (NADES12 extracts).
These samples were compared with aqueous ethanolic solution extracts of Fedora 17 and Futura 75. To determine the intra-variety differences between the control and NADES extractions, one-way ANOVA, Tukey's test, and p<0.05 were used.

UHPLC tandem mass spectrometry summary identification of phytochemicals in hemp microgreen extracts

Target and suspect MS/MS analysis were performed usnig a Product Ion Scan (PIS) method. Identification of molecules was performed by comparing the MS/MS ion spectra with the fragmentation data available in online libraries (MzCloud, Mass Bank Europe). Rutin, p-cumaric acid and ferulic acid were identified using the standard

	Compounds	RT	[M-H]$^-$ (m/z)	MS^2	Acqueous Ethanolic extracts CH3OH/H2O (80:20 v/v) STD	Fedora 17	Futura 75	Nades 11 L-proline +Acetic a. + water Fedora 17	Futura 75
1	Dihydroxybenzoic acid-O-hexos	4.16	315	152-109-108		x	x	x	x
3	p-cumaric acid	6.24	163	93-119-117-65-91	x	x			
4	Ferulic acid	6.34	193	134-132-149	x	x		x	
7	Rutin	6.69	609	300-299-301	x			x	
6	Vitexin	6.75	431	283-311-277				x	x
5	Kaempferol-3-O-glucoside	6.84	447	270-284-285-268-174		x	x	x	x
9	Luteolin	7.94	285	241-175-199- 151- 256		x		x	
8	Acacetin	8.11	283	239-283		x	x	x	x
9	Cannabisin B	8.16	595	432-595-485-269-322		x		x	x
10	Grossamide	9.01	623	460-428		x	x		
11	Cannaflavin B	10.95	367	352-296-334-309-269		x	x	x	x
12	Cannaflavin C	12.74	435	351-309-297-337		x	x	x	x
13	Canniprene	14.65	341	209-163-259				x	x



Chromatograms of **Rutin** (right) and **Canniprene**(left) detected in Nades 11 extracts