Deliverable title	**D3.2 Lyophilized biomass from cultivated thistles**
Deliverable Lead:	ISA-CM
Related Work Package:	**WP3 Sustainable cultivation of thistles**
Related Task:	Task 3.7 Lyophilization of sampled cultivated thistles
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Dissemination level	Public
Due Submission Date:	30.11.2020 (month 19)
Actual submission:	30.11.2020
Start date of project	**01.05.2019**
Duration	36 months (after project end extension: 48 months)
Abstract	Cultivated thistles (tubular flower collected from thistle flower heads) have been used to prepare crude extracts by maceration; the latter were hence lyophilized and stored at room temperature prior to use.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/06/2021	Bouthaina Dridi Al Mohandes	First version
V2.0	17/04/2023	Bouthaina Dridi Al Mohandes	Comments after peer reviewing process

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1. Lyophilization of tubular flowers from cultived thistles

1.1 Italian site

1.1.1 *Onopordum tauricum (ecotype Marche)*

Tubular flowers from approximately 1244.1 g flower heads (Figure 1a) collected in June -July 2020 from 22 plants of *Onopordum tauricum* ecotype Marche cultivated at the Italian site (date of transplanting of the young plants: 02.04.2020) were manually separated from receptacle, immediately after harvesting and collected at freezing temperature prior to the lyophilization process.
The freeze-dried enzymatic extract was obtained by following the procedure described by Tejada & Fernández-Salguero (2003).
Briefly, 143 g of *O. tauricum* tubular flowers were macerated in demineralized water (1:10 w/v) for 24 hours at 4 °C. The liquid phase was recovered by filtration through a muslin cloth and subsequent centrifugation (5000 g, 10 min). Finally, the aqueous crude extract (CE) was freeze-dried and stored at -20°C until it was used. As a result, 5.38 g of dry extract/100 g of fresh flowers was obtained (Table 1).

1.1.2 *Onopordum tauricum (ecotype Umbria)*

Tubular flowers from approximately 4714.5 g flower heads (Figure 1b) collected in June -July 2020 from 38 plants of *Onopordum tauricum* ecotype Umbria cultivated at the Italian site (date of transplanting of the young plants: 02/04/2020) were manually separated from receptacle, immediately after harvesting and collected at freezing temperature prior to the lyophilization process.
The freeze-dried enzymatic extract was obtained by following the procedure described by Tejada & Fernández-Salguero (2003).
Briefly, 101 g of *O. tauricum* tubular flowers were macerated in demineralized water (1:10 w/v) for 24 hours at 4 °C. The liquid phase was recovered by filtration through a muslin cloth and subsequent centrifugation (5000 g, 10 min). Finally, the aqueous crude extract (CE) was freeze-dried and stored at -20°C until it was used. As a result, 7.32 g of dry extract/100 g of fresh flowers was obtained (Table 1).

Table 1. Overview of the lyophilized biomass from cultivated thistles of *O.tauricum* from Marche and Umbria respectively.

Extracts	**Cultivated *O. tauricum* ecotype Marche**		**Cultivated *Onopordum tauricum* ecotype Umbria**	
Flowers weight	143,6g	25g	101 g	25g
Date for the lyphilizatiopn	03/09/2020	29/09/2020	30/07/2020	29/09/2020
Weight of aqueous extract (g)	1137.76	254.63	1003	254.38
Lyophilized extract weight (g)	5.38	0.92	7.32	1.83

a

b

Figure 1: Flower heads of *Onopordum tauricum* from Marche (a); flower heads of *Onopordum tauricum* from Umbria (b).

1.1.3 *Onopordum platylepis*

In June -July 2020, no flower heads could be collected from the 72 cultivated plants of *O. platylepis,* since these plants have not differentiated the stem with the flowering branches.

1.1.4 *Cynara humilis*

Tubular flowers from approximately 57 flower heads collected in June -July 2020 from 46 plants (out of the total 72 transplanted plants): the difference are the plants that have survived the phenological rosette stage, i.e. they have not differentiated the stem with the flowering branches) of *Cynara humilis* cultivated at the Italian site (date of transplanting of the young plants: 02/04/2020) were manually separated from receptacle, immediately after harvesting and collected at freezing temperature prior to the lyophilization process.
The freeze-dried enzymatic extract was obtained by following the procedure described by Tejada & Fernández-Salguero (2003).

1.2 Tunisian site

At the Tunisian site, cultivation trials have been started with a delay in September 2020.

1.2.1 *Onopordum tauricum*

Tubular flowers collected in July 2021 from 7 transplanted plants were manually separated from receptacle immediately after harvesting and then were stored at freezing temperature (- 80°C) for further analysis and lyophilization.

1.2.2 *Onopordum platylepis*

Tubular flowers collected in July 2021 from 60 transplanted plants (originating from three different ecotypes) were manually separated from receptacle immediately after harvesting and then were stored at freezing temperature (- 80°C) for further analysis and lyophilization.

Fig1. Flower heads of cultivated *Onopordum platylepis*

Fig 2. Tubular flowers separated from the receptacle of *O.platylepis*

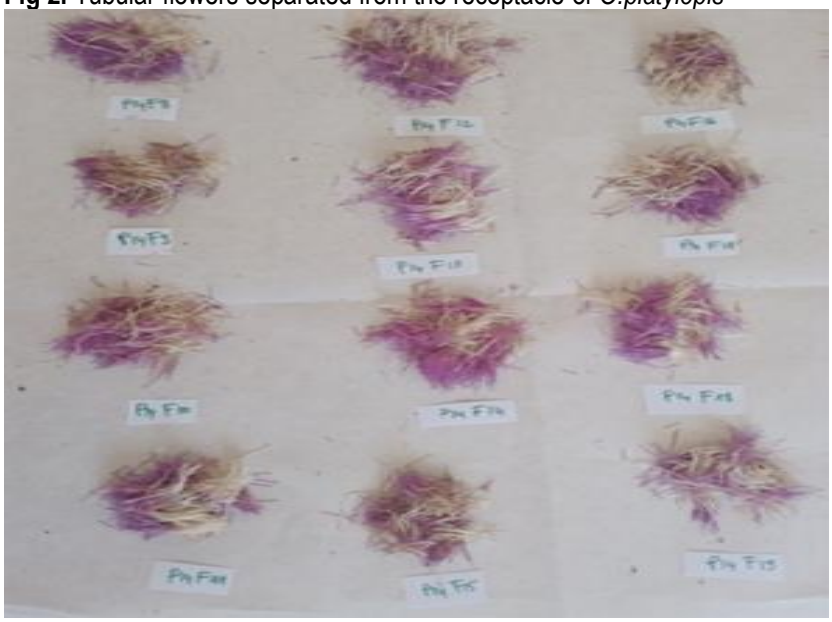

Fig 3. Tubular flowers obtained from each flower head of cultivated *O.platylepis*

	Cultivated plants of *O.platylepis*		
	Average	Min	Max
Average weight of flower heads (g)	29,17 (±1,30)	12,97	37,69
Average weight of fresh flower tubules (g flower head-1)	6,99 (±0,39)	3,62	9,06
Average weight of dry flower tubules (g flower head-1)	2,25 (±0,10)	1,25	3,12

1.2.3 *Cynara humilis*

In July 2021, no flowers could be harvested from the cultivated *Cynara humilis* plants because these plants did not develop a main stem or lateral shoots

Fig 4. Cultivated *Cynara humilis*