

Freeze Drying of food samples prior to Pressurized Solvent Extraction

Lyovapor™ L-200 Pro, SpeedExtractor E-914, Mixer B-400, Multivapor P-6

1. Introduction

In this Short Note, we highlight the freeze drying (lyophilization) and subsequent fat extraction of food samples. The SpeedExtractor E-914 / E-916 can be used only for solid or semi-solid samples. Residual moisture needs to be removed by mixing the sample with a drying agent such as diatomaceous earth. This can be omitted by using freeze drying: the drying is initiated mainly by direct sublimation of ice at low temperatures. In this study, chicken breast meat, hen's egg and basa fish meat were chosen as examples. The freeze dried samples were extracted using the SpeedExtractor E-914 and the obtained fat contents were compared to established literature values.

2. Experimental

All the chosen samples were homogenized for a few seconds using the Mixer B-400. The fresh chicken breast meat and basa fish samples were cut into pieces prior to mixing. One set of triplicate samples was prepared for the fat extraction, a second set of triplicate samples was prepared for the determination of the moisture content.

The weights of the homogenized samples, shown in Figure 1 (a, c, e), were recorded for later fat determination. All samples were placed on a plastic dish and frozen for 24 hours in a deep freezer at -24°C.

After 24 hours of deep freezing, the samples were transferred with the plate into the Lyovapor™ L-200 Pro for freeze drying. In Table 1, the Lyovapor™ L-200 Pro general settings are presented.

Table 1: General settings for drying in Lyovapor™ L-200 Pro.

Drying chamber	Sample collapse temperature [°C]	Safety temperature below collapse [°C]	Gas type
Heatable shelves	Inactivated	Inactivated	Ambient air

The steps of the primary and secondary drying process were programmed on the Lyovapor™ L-200 Pro as listed in Table 2. The parameters can also be programmed on the computer using the Lyovapor™ software.

Table 2: Parameters using Lyovapor™ L-200 Pro.

Step	1	2
Phase	Primary Drying	Secondary Drying
Time [hh:mm]	13:00	4:00
Temperature set point [°C]	15.0	15.0
Temperature gradient [°C/min]	1.00	1.00
Pressure type	Regulated	Regulated
Pressure set point [mbar]	0.300	0.269
Safety pressure [mbar]	1.50	1.50
Safety pressure duration [sec]	10	10

The freeze dried samples, shown in Figure 1 (b, d, f), were homogenized using a mortar, transferred quantitatively into a weighing boat and weighed. The sample was mixed with

10 g of diatomaceous earth and transferred into an 80 mL extraction cell of the SpeedExtractor E-914 [1].

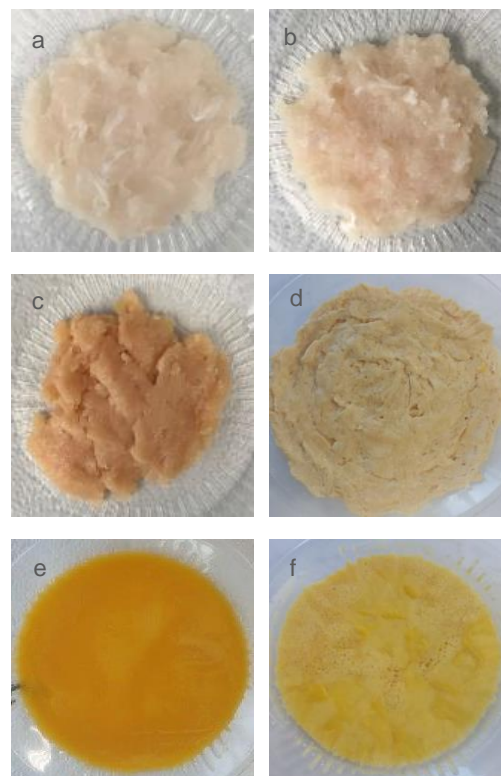


Figure 1: Fresh homogenized fish (a), chicken (c) and egg (e) samples before and after Freeze Drying (b, d, f).

The extraction of the freeze-dried samples was performed using the parameters listed in Table 3. Pre-weighed 240 mL vials were used to collect the extracts.

Table 3: Parameters using SpeedExtractor E-914 [1].

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	Dichloromethane: Hexane 50% : 50%
Cells	80 mL
Vials	240 mL
Cycles	3
- Heat-up	5 / 1 / 1 min
- Hold	10 / 10 / 10 min
- Discharge	4 / 4 / 4 min
Flush with solvent	2 min
Flush with gas	4 min
Total extraction time	1 h 19 min

The extracts were dried using the Multivapor P-6 with the parameters listed in the Table 4, then placed in a drying oven for 30 min at 102 °C. The extracts were cooled down to ambient temperature in a desiccator for 60 min and the weight was determined.

Table 4: Parameters using Multivapor P-6.

Parameter	Value
Temperature rack	55 °C
Temperature chiller	10 °C
Rotation	Level 5
Step 1	
- Pressure start	800 mbar
- Pressure end	750 mbar
-Time	30 min
Step 2	
- Pressure start	500 mbar
- Pressure end	250 mbar
-Time	25 min
Step 3	
- Pressure start	250 mbar
- Pressure end	250 mbar
-Time	10 min
Total time	65 min

The freeze-dried triplicates for the moisture content determination were dried in a drying oven for 120 min at 105 °C, cooled down in a desiccator for 30 min and weighed [3]. The difference to the initial weight was defined as moisture content.

3. Results and Discussion

The determined moisture contents of the freeze dried triplicate samples are shown in Table 5. The results correspond well to the expected range of 1 – 5 % [2].

Table 5: Moisture contents of freeze dried samples.

Sample	Moisture content [%]	Average [%]
Chicken 1	3.51	
Chicken 2	3.54	
Chicken 3	3.49	3.51
Egg 1	3.26	
Egg 2	3.12	
Egg 3	3.49	3.29
Fish 1	2.82	
Fish 2	3.13	
Fish 3	3.28	3.08

The results of the fat extraction of the freeze dried samples using SpeedExtractor E-914 are shown in Table 6. They corresponded well with the expected fat content mentioned in literature [4, 5].

Table 6: Extracted fat using SpeedExtractor E-914.

Sample	Weight of fresh sample [g]	Weight of freeze-dried sample [g]	Fat content of fresh sample [%]	Average [%] (RSD)	Fat content in literature [%]
Chicken 1	40.8076	12.1583	1.34		
Chicken 2	40.8523	11.9862	1.39		
Chicken 3	40.8294	12.0641	1.40	1.38 (2.50)	1.50 [4]
Egg 1	11.3344	2.9799	9.42		
Egg 2	11.4564	2.9977	9.27		
Egg 3	11.4835	3.0946	9.64	9.44 (1.96)	10.30 [4]
Fish 1	37.3456	4.9986	3.50		
Fish 2	37.5102	4.9865	3.62		
Fish 3	37.6297	5.1248	3.57	3.56 (1.69)	3.98 [5]

4. Conclusion

With the Lyovapor™ L-200 Pro, a high drying efficiency was achieved. The analyzed samples of chicken, egg and fish had a residual moisture content of 3.51 %, 3.29 % and 3.08 % (n=3) after freeze drying which corresponds well to the typical range of 1 – 5 % for freeze dried biological samples mentioned in literature [2]. The initial moisture content of the samples was 70.44 % for chicken, 73.53 % for egg and 86.57 % for fish (n=3). Hence, the freeze drying method on the Lyovapor™ L-200 Pro can lead to an average moisture removal of 96 – 97 %. In summary, the Lyovapor™ L-200 Pro enables to dry food samples such as chicken, fish and egg quickly and effectively prior to Pressurized Solvent Extraction.

After freeze drying, the samples were extracted using the SpeedExtractor E-914 and the fat content of the original samples was determined: 1.38 % for chicken, 9.44 % for egg and 3.56 % for fish (n=3). The obtained fat contents are comparable to reference values in literature [4, 5].

5. References

- [1] BUCHI Application Note, No.069/2012, Extraction of PCDD/Fs and PCBs in fish using SpeedExtractor E-914.
- [2] FDA Guideline for the Determination of Residual Moisture in Dried Biological Products, 89D-0140. US Food and Drug Administration.
- [3] Determination of Moisture in Foods, GB 5009.3-2016. Chinese National Food Safety Standard.
- [4] Swiss Federal Food Safety and Veterinary Office. <http://www.naehrwertdaten.ch> (accessed Apr 10, 2017).
- [5] United States Department of Agriculture, Food Composition Databases. <https://ndb.nal.usda.gov/ndb> (accessed Apr 12, 2017).