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Professional paper

VARIATION IN FAT AND PROTEIN CONTENT AT ANISE LANDRACES (*PIMPINELLA ANISUM* L.) STORED AT DIFFERENT TEMPERATURES AND PACKAGE

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Abstract

Anise (*Pimpinella anisum* L.) is an aromatic plant from the Apiaceae family which have various medical properties. It has been used to protect human health and improve the quality of human life for thousands of years. This plant has different benefits and uses such as reducing the symptoms of depression, being rich in nutrients, protecting against gastric ulcers, preventing the growth of fungi and bacteria, helping relieve menopause symptoms, balancing blood sugar levels and blood pressure, reducing inflammation, stimulant, culinary significance, skin benefits. Numerous studies show that wild and cultivated *P. anisum* L. has a wide range of compounds, including flavonoids, terpenes, and essential oils, they are rich also in bioactive compounds, such as phenols, tannins, carotenoids, and fatty acids. These compounds have pharmacological activities, antioxidant, antibacterial, antifungal, insecticidal, antiviral, anti-inflammatory, analgesic, gastro-protective, and antidiabetic activities. In this research, Pimpinella anisum L was investigated from 11 different localities in terms of latitude and longitude, in the town of Negotino, Republic of North Macedonia. The nutritive traits of *Pimpinella anisum* L were analysed 15 days before harvest and 3 months after storage at room temperature, under +4oC, and at -18oC and it was observed that the mean value of protein content was 17.83%; 17.62%; 17.40%; 17.25% and the mean value of fat content was 16.23%; 16.23%, 15.88%; 15.76%, respectively. The samples were stored in a zip plastic bag (manually compressed air).

Keywords: Pimpinella anisum, nutritive traits, aromatic plant, compounds, temperature

Introduction

Pimpinella anisum L. (common names aniseed or anise) is an annual herbaceous plant that belongs to the *Umbelliferae* family. This plant reaches a height of 30-50 cm and has white flowers and small green to yellow seeds, it is a plant that can be found in different parts of the world such as in Western Asia, Europe, Turkey, Iran, India, Egypt and other places (Shojaii & Abdollahi Fard, 2012; Gülçın et al., 2003; Giordano et al., 2022; Moazzami Farida et al., 2020). Some plants grow in dry rocky places, rock crevices, meadows, and mountain pastures (Nasır & Yabalak, 2021). For thousands of years, this plant has been used to maintain human health and increase the quality of life (Soussi et al., 2023). The seeds of this plant are used in traditional medicine for the treatment of different diseases such as analgesic, carminative, aromatic, muscle relaxant, antiseptic, disinfectant, and diuretic. The treatment of those diseases is due to its antimicrobial and antioxidant properties (Ghlissi et al., 2020; Soltani et al., 2021; Nasır & Yabalak, 2021). *P. anisum* possesses biological, pharmacological, and chemical properties (Nasır & Yabalak, 2021). In various scientific reports, it has been reported that *P. anisum* has various therapeutic properties, such as digestive, gynecological, neurological, and respiratory disorders (Gülçın et al., 2003; Mahboubi & Mahboubi, 2021).

It is a plant rich in bioactive substances with properties like anti-diabetic, anti-inflammatory, and gastric diseases. Extracts obtained from the leaves and seeds of this plant have shown strong properties against fungal, bacterial, and viral pathogens (Soussi et al., 2023). The antibacterial activity of the oils obtained from Anise was determined against Gram-negative and Gram-positive bacteria and yeast (Nasır & Yabalak, 2021). This plant has shown also antioxidant, anticonvulsant, analgesic, gastro-protective (Sun et al., 2019).

The seeds that grow from this plant are used in food for flavoring due to the aromatic compounds, while the essential oils and extracts are used in perfumery, medicine, and other industrial purposes (Gülçın et al., 2003; Das et al., 2021; Jafari et al., 2022). The fruits from Pimpinella anisum L., are rich in essential oil (1.5–6%) (Reineccius, 2013) where the major component is trans-anethole (75–90%) other constituents include different groups of chemical compounds such as proteins, cellulose fibers, carbohydrates, coumarins, lipids and flavonoids (Sun et al., 2019). In the extract from this plant were identified estragole, methyl chavicol and anisaldehyde, scopoletin, umbelliferone, sterols, and terpene hydrocarbons (Ghlissi et al., 2020). According to Taddeo et al., (2017), umbelliprenine was found to be the major prenyloxy secondary metabolite, while Orav et al., 2008 found as the main compound in the EOs was trans-anethole, the other copmpound present in small percentages such as γ-himachalene, trans-pseudoisoeugenyl 2-methylbutyrate, p-anisaldehyde and methylchavicol. Saibi et al., 2013 found also that the main compound in the EOs were trans-anethole, estragol, o-isoeugenol and γ-himachalene. The natural compounds found in essential oils have acaricidal activity. Terpenoids are compounds of essential oils, that have insecticidal and acaricidal activity (El-Sayed et al., 2022), EOs have also antioxidant, and antiviral activity (Azam et al., 2023). P. anisum seeds are used for milk production. In the research done by Hosseinzadeh et al., 2014 for P. anisum aqueous and ethanolic extracts show that the milk production was increased significantly.

The aim of this study was to do analyses of nutritional properties for the Pimella anisum L. that were collected from different places.

Materials and Methods

Plant material: Plants of Pimpinella anisum were collected from different places in the town Negotino, Republic of North Macedonia. Table 1 gives details about the places where the materials were collected.

Table. 1 Name of the places, latitude, longitude and elevation from where the raw materials of *Pimella anisum* L. are collected

Local name / Abbreviation	**Place / Name of locality**	**Latitude**	**Longitude**	**Elevation (a.s.l.)**
Timjanski / Tm	Negotino, v. Timjanik	41°28'09"N	22°04'54"E	212m
Przdevski / Pz	Negotino, v. Przdevo	41°25'17"N	22°09'46"E	218m
Dolno Disanski / DD	Negotino, v. Dolni Disan	41°25'44"N	22°05'55"E	288m
Gorno Disanski / GD	Negotino, v. Gorni Disan	41°23'30"N	22°06'33"E	721m
Pepelishki / Pl	Negotino, v. Pepelishte	41°31'29"N	22°07'49"E	521m
Tremnichki / Tr	Negotino, v. Tremnik	41°26'15"N	22°09'13"E	845m

Vojshanski / Vo	Negotino, v. Vojshanci	41°28'25"N	22°09'48"E	535m
Veshjanski / Ve	Negotino, v. Vejshe	41°22'58"N	22°08'14"E	833m
Janoshevski / Jn	Negotino,v. Janoshevo	41°37'51"N	22°01'01"E	420m
Dubrovski / Db	Negotino, v. Dubrovo	41°28'43"N	22°08'12"E	376m
Marenski / Mr	Negotino, v. Marena	41°28'11"N	22°01'59"E	630m

Nutrient Analyses: The raw material was cleaned and dried, therefore according to the specification in Ph. Eur. 5, the presence of moisture in the plant does not need to be more than 8.0 %. The samples were analysed for moisture, carbohydrate, protein, fat, ash, and crude fiber content in the state phytosanitary laboratory. All the analyses were done based on accredited methods (http://www.iarm.gov.mk/files/Akreditirani-tela/Laboratorii/OB05-25_LT-036.pdf) verified by the Institute for Accreditation of the Republic of Macedonia. Nutritive traits of *Anisum pimpella* L. were done in different conditions, such as analyses done after 15 days of harvest and before storing, stored for 3 months at room temperature in zip plastic bag (manually compressed air), stored for 3 months at refrigerator +4°C zip plastic bag (manually compressed air), stored for 3 months froze at -18°C zip plastic bag (manually compressed air), in %. Besides those analyses, fluctuation in moisture content, carbohydrate content, protein content, fat content, ash content, and crude fiber content depends on the temperature regime of *Pimpella anisum* L. seed storage, in %.
The total protein content was measured using the Kjeldahl method, this method determines the total nitrogen content and is used to measure the protein content (Varelis, 2016). The fat content was extracted by Soxhlet extraction. The determination of fibres was performed according to the Kürschner-Hanak method, therefore the moisture content was measured following the drying method for plants, and the plant material was dried at 105 ± 2°C to constant weight. The carbohydrate content can be measured when you calculate the percentages remaining after all other components have been measured. The total ash content is determined by equalling the weight of the ash and then dividing it by the weight of the original sample multiplied by 100%. More details about the procedure of the adopted methods are written in the following scientific articles (Ghlissi et al., 2020; Balkhyour et al., 2021; Hozzein et al., 2020).

Results

After 3 months of storage at room temperature (app. 20-25°C), the content of the average HW drops significantly and ranges from 11.30% in Ve landrace to 14.90% in Mr landrace. Almost approximate values with a minimal increase were measured for fennel seeds stored for 3 months at +4°C, with the lowest average moisture in Ve landrace (11.40 %), and the highest average in the same Mr landrace (15.00 %). Even in the case of fennel seeds kept frozen for 3 months at -18°C, a higher average HV can be observed, from 12.00 % in Pl landrace to 15.50 % in Mr landrace (Table 2, 3, 4, and 5).
In terms of total carbohydrates (TC) determined 15 days after harvest, the seed of Tr landrace is the lowest (41.13 %), and that of Pr is the highest (45.47 %). In all other storage conditions, TC was found to be the least at We Landrace 36.58% (stored for 3 months at room temperature), with a minimum drop stored for 3 months at +4°C (35.90%) and at -18°C (35.70%). In the same landrace (GD), the highest content of TC was found and

it was stored for 3 months at room temperature (45.41%), at +4°C (44.80%) and at -18°C (44.70%) (Table 2, 3, 4 and 5).

The protein content in anise seeds analyzed 15 days after harvest ranges from 16.80 in Db landrace to 18.90% in Jn, where even after 3 months of storage at room temperature, the highest content is 18.80% (Jn), while at Vo it is the lowest 16.70% as well as after 3 months of storage at +4 ºC (16.60%). The same values and rank were found when stored in a freezer at -18°C (16.00% for Db and Tr to 18.00% for Ve, i.e. for Ve and GD kept at +4°C) (Table 2, 3, 4, and 5).

There is no great reduction in the fat content as a result of the different temperature regimes, some of which are inappropriate. The lowest content 15 days after harvesting activities was found in Pr landrace (15.30%), after 3 months of storage at room temperature, they were minimally reduced in Vo landrace, where the reduction continues and was recorded as the lowest even at +4°C and unchanged at - 18°C (14.60%). The highest content at all temperature regimes and storage methods (Tandoh et al., 2017) is recorded for Mr. Landrace, namely 17.50% 15 days after harvest and 3 months stored seed at room temperature, i.e. 17.10% placed in a refrigerator (at +4°C) and 17 .00% in a freezer (at -18°C). The immobilization of fats is longer lasting and the last in the series of hydrolytic processes (Piršelová and Matušíková, 2013), which is why there is a very small difference in the values at different storage temperatures (Table 2, 3, 4, and 5). Very small oscillations in the CF content were obtained with the analyzed samples of anise seed material, so that in Gd landrace, 15 days after harvest and stored for 3 months at room temperature were the lowest, respectively (11.40%; 11.10%), while in Tr landarce it is the highest 15 days after harvest (17.30%), and after 3 months stored at room temperature it decreases (16.60%). In Tm landrace, the values stored in the refrigerator and freezer are the lowest (11.00%), while in Jn it is significantly lower with a continuous decrease in the content during storage at +4 ºC (15.90%) and in the freezer (15.70%) (Table 2, 3, 4 and 5).

Ash, which represents the mineral component in the studied anise landrace, is represented in the lowest content in GD measured 15 days after harvest (4.43%), in Jn after 3 months kept at room temperature and at +4°C, respectively (8.55%; 11.00%) and at -18°C almost the same value as in a refrigerator (10.90%). In the same landrace in Tr grown at 845 m a.s.l. has the highest ash content after harvest, 3 months at room temperature and stored in a refrigerator (8.97%; 15.62%, 17.30%), while Tm anise seed has 15.90% ash, which represents a high content (Table 2, 3, 4 and 5).

Table 2. Nutritive traits of anise landraces after 15 days of harvest and before storing, in %

Landraces	**Moisture***	**Total carbohydrate**	**Protein**	**Fat**	**Crude fiber**	**Ash (100-o.m.)**
Tm	15.90	44.23	18.2	15.8	13.8	7.97
Pz	14.70	45.47	17.80	15.30	13.60	7.83
DD	15.50	43.15	17.90	16.30	14.90	7.75
GD	14.20	49.87	18.60	15.70	11.40	4.43
Pl	13.80	42.02	18.10	16.90	15.60	7.38
Tr	14.90	41.13	17.20	15.40	17.30	8.97
Vo	15.30	45.44	16.90	15.10	16.30	6.26
Ve	14.40	41.98	18.50	16.60	16.50	6.42
Jn	16.70	42.36	18.90	16.90	17.10	4.74
Db	15.80	43.18	16.80	16.60	15.70	7.72
Mr	15.50	42.65	17.60	17.50	16.10	6.15
x±Sx	**15.15±0.06**	**43.77±0.06**	**17.83±0.04**	**16.23±0.05**	**15.45±0.12**	**6.87±0.21**

Table 3. Nutritive traits of anise landraces stored for 3 months at room temperature in zip plastic bag (manually compressed air), in %

Landraces	Moisture*	Total carbohydrate	Protein	Fat	Crude fiber	Ash (100-o.m.)
Tm	13,10	40,11	18,00	15,8	11,20	14,89
Pz	13,20	40,99	17,00	15,30	12,40	14,31
DD	14,30	39,82	17,60	16,30	13,60	12,68
GD	12,5	45,41	18,10	15,70	11,10	9,69
Pl	11,60	39,22	18,00	16,90	14,60	11,28
Tr	12,80	36,78	17,00	15,40	15,20	15,62
Vo	12,50	41,25	16,70	15,10	15,60	11,35
Ve	11,30	36,58	18,30	16,60	16,10	12,42
Jn	14,40	39,15	18,80	16,90	16,60	8,55
Db	13,50	39,23	16,80	16,60	15,40	11,97
Mr	14,90	38,71	17,50	17,50	15,80	10,49
x±Sx	**13,16±0.09**	**39,75±0.06**	**17,62±0.04**	**16,23±0.05**	**14,33±0.14**	**12,11±0.18**

Table 4. Nutritive traits of anise landraces stored for 3 months at refrigerator +4°C zip plastic bag (manually compressed air), in %

Landraces	Moisture*	Total carbohydrate	Protein	Fat	Crude fiber	Ash (100-o.m.)
Tm	13,30	39,80	17,80	15,80	11,00	15,60
Pz	13,20	40,60	17,00	15,10	12,10	15,20
DD	14,50	39,10	17,50	16,10	13,20	14,10
GD	12,4	44,80	18,00	15,50	11,10	10,60
Pl	11,60	38,70	17,40	16,70	14,00	13,20
Tr	12,80	36,20	17,00	15,10	14,40	17,30
Vo	12,60	40,30	16,60	14,60	15,20	13,30
Ve	11,40	35,90	18,00	16,20	15,70	14,20
Jn	14,30	38,70	18,10	16,30	15,90	11,00
Db	13,50	37,40	16,80	16,20	15,10	14,50
Mr	15,00	36,50	17,20	17,10	15,30	13,90
x±Sx	**13,22±0.09**	**38,91±0.07**	**17,40±0.03**	**15,88±0.05**	**13,91±0.13**	**13,90±0.14**

Table 5. Nutritive traits of anise landraces stored for 3 months as freezed at -18°C zip plastic bag (manually compressed air), in %

Landraces	Moisture*	Total carbohydrate	Protein	Fat	Crude fiber	Ash (100-o.m.)
Tm	13,50	39,70	17,80	15,60	11,00	15,90
Pz	13,90	40,60	17,00	15,00	12,00	15,40
DD	14,70	39,10	17,40	16,00	13,00	14,50
GD	12,8	44,70	17,90	15,50	11,00	10,90
Pl	12,00	38,70	17,20	16,50	13,90	13,70
Tr	13,10	36,20	16,60	15,10	14,30	17,80
Vo	12,90	40,10	16,30	14,60	15,20	13,80

Ve	12,10	35,50	18,00	16,00	15,70	14,80
Jn	14,50	38,70	17,80	16,10	15,60	11,80
Db	13,90	37,40	16,60	16,00	15,00	15,00
Mr	15,50	36,30	17,10	17,00	14,80	14,80
x±Sx	**13,61±0.08**	**38,82±0.07**	**17,25±0.03**	**15,76±0.04**	**13,77±0.13**	**14,40±0.13**

Discussion

Quality properties and the changes that occur with storage are difficult to predict, especially when stored under different temperature regimes because temperature is a driver of metabolic functions and changes in nutrients (Giannou and Tzia, 2007). Although it is considered that a temperature regime of storage of 20-25 ºC is favorable, and anything above 25 ºC causes aging of the seed accompanied by hydrolytic processes and a decrease in nutritional properties, however, long-term storage of 3 months influences the overall nutritional value and quality of the stored anise seed (Bewley et al., 2013). One of the main drivers of enzyme activity is the water content on and in the seed because drying prevents seed germination and quality losses (McDonald, 2007). Therefore, great attention is paid to the ambient relative humidity (RH), the content of hygroscopic moisture, and the temperature of seed storage (Afzal et al., 2017). The hygroscopic moisture (HW) was the highest average measured after 15 days of harvest before storage of anise seed material with an average value of all examined anise landraces 15.15±0.06. From this, it can be concluded that the seeds stored for 3 months at room temperature have the lowest HG, although the contents are minimally increased under the conditions of storage for 3 months at +4°C and -18°C, which means that this parameter, storage in a refrigerator or freezer is recommended (Abebaw et al., 2016) (Figure 1a).

The constant drop in the average TC content of anise seeds starting from the measured 15 days after harvest (43.77±0,06) when it is the highest, with a decrease during storage for 3 months at room temperature (39.75±0,06) is completely understandable, at +4°C (38.91±0,07) and at -18°C (38.87±0,07). Namely, the seed breathes and consumes from reserve carbohydrates, and therefore the obtained values are expected (Figure 1b).

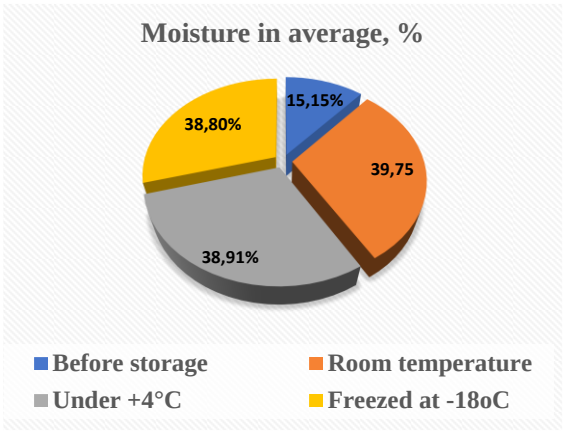
When the seed is stored, it breathes and consumes not only carbohydrates (glucose) but also other nutrients such as proteins and fats, which go through conversion processes to be used in cellular respiration (Chandra et al., 2007) (Figure 2). Most of the proteins present in mature seeds have structural, but if necessary, metabolic roles, so the seeds contain different amounts of proteins, especially if the seed material is stored in unfavorable conditions that can serve to provide amino acids during seed germination. and seedling growth (Krishnan and CoeJr, 2001). Therefore, the average content of proteins and fats during storage of anise seeds decreases gradually (Shewry et al., 1995), but continuously, from 17.83±0.04 when measured 15 days after harvest, 17.62±0.04 when stored for 3 months at room temperature, 17.40±0.03 for that kept for 3 months at +4°C reaches 17.25±0.03 for the seed kept for 3 months at -18°C. The dynamics of the average fat content of stored fennel seeds are very similar. Where, after 15 days of harvest management (Hengsdijk and de Boer, 2017), the fat content was determined, the highest value of 16.23 ±0.05 was found, which was unchanged even after 3 months of storage at room temperature, but decreased when stored for 3 months at +4°C (15.88±0,05) and at -18°C, although minimal (15.76±0.04). If storage at room temperature is continued, the fat content will drop drastically due to their involvement in seed metabolism and cellular respiration (Miart et al., 2014), but it will also lead to oxidative changes and its unusability accompanied by an unpleasant smell, therefore it is

considered that storage in a refrigerator or freezer is most suitable with minimal losses in terms of fat (Mesele et al., 2022) (Figure 1c, d).

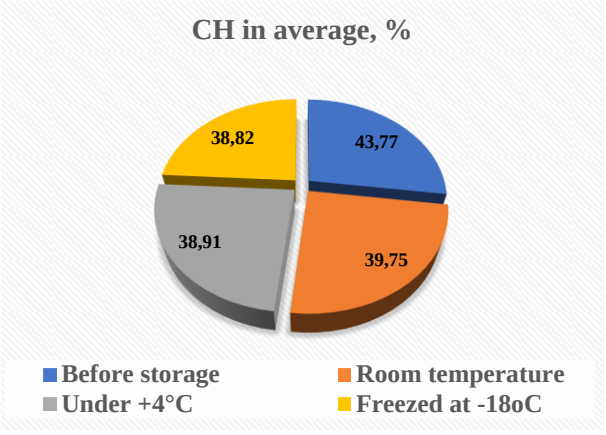
The highest average content of CF was recorded 15 days after the harvest of anise seed (15.45±0.12), so after 3 months of storage at room temperature it dropped significantly (14.33±0.11). Even in a refrigerator kept for 3 months, the seeds have a low CF content (13.91±0.13), that is, a little lower when stored at -18°C (13.77±0.13). This dynamic indicates the fact that by drying and keeping at room temperature, and in the seed coat, which is structured by a network of macromolecular polymers (Cosgroveand Jarvis, 2012; Burton et al., 2010), mainly polysaccharides of the type cellulose, hemicellulose, pectin (Zykwinska et al., 2005) which during the accumulation of organic matter are partly stored in the apoplast, that is, the barrier called the cuticle (Lafon-Placette and Kohler, 2014). But when stored at an inappropriate temperature (20-25C) they are hydrolyzed (Bouaziz et al., 2020), and in this case it is minimal. Hence, the observed dynamics are expected, but it was prevented by storing the seed material at -18°C (Figure 1e).

The increase in ash content means a decrease in organic matter, so if 15 days after harvest the average value for the analyzed anise landraces is only 6.87±0.21, already after 3 months of storage at room temperature it shows almost twice as high content (12.11±0,18). This only points to the fact that the seed, as a living plant organ, performs its metabolic functions, consuming organic matter, which is especially noticeable with the doubled content after 3 months of storage at room temperature, which supports the inappropriateness of the temperature regime. By placing it in the refrigerator and freezer, the metabolic changes that have started are reduced to a minimum. Therefore, it is generally recommended to store the seed material in a refrigerator or freezer, but after previously achieving hygroscopic moisture not higher than 16% (limit seed moisture for laboratory determination of seed germination, otherwise the freezing of free water and the formation of crystals will occur, which will cause mechanical damage to the embryo, and with this will render the seed incapable of germinating and producing a new plant (Figure 1f).

a)



b)



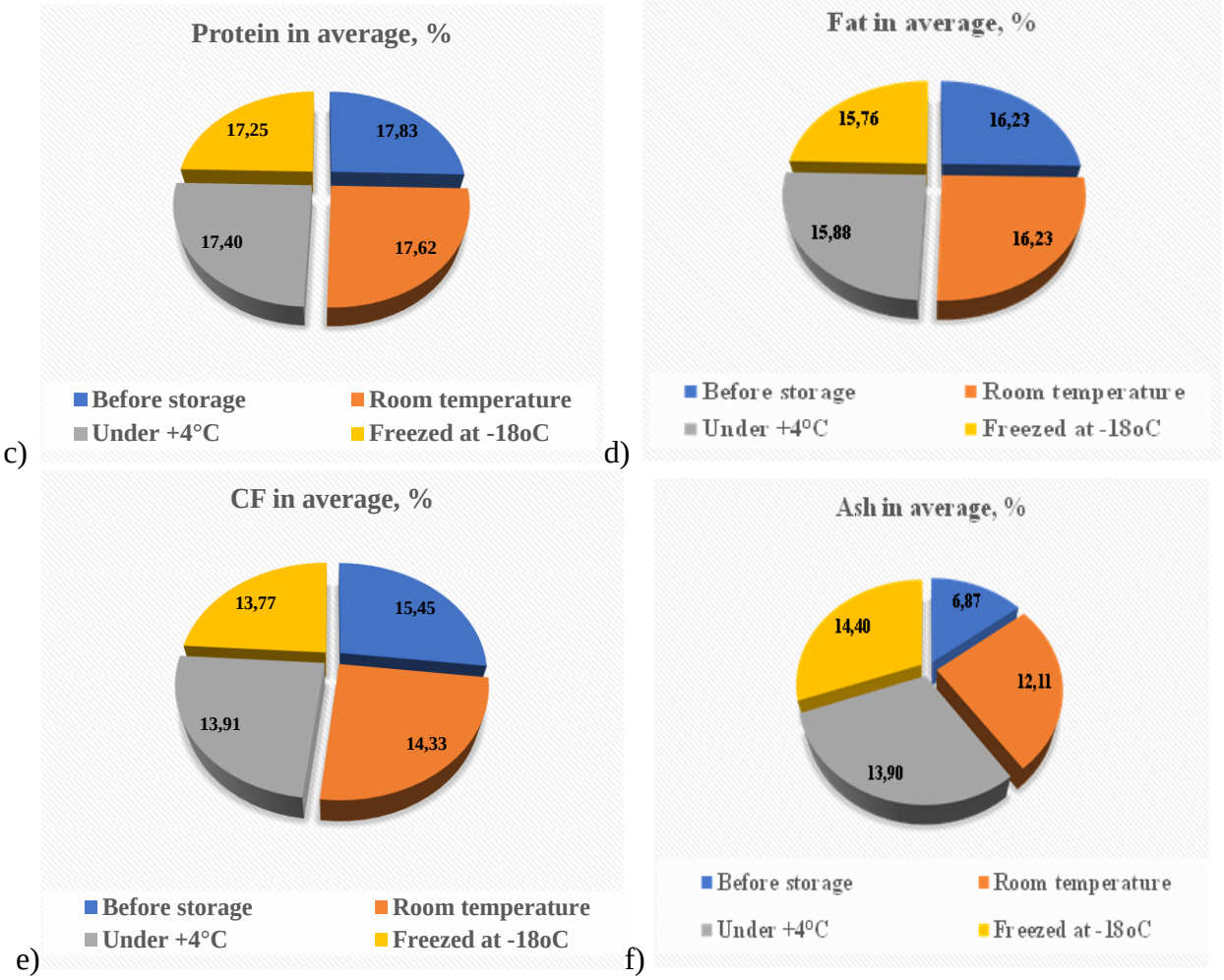


Figure 1. Fluctuation in a) an average moisture content, b) carbohydrate (CH), c) protein, d) fat, e) crude fiber (CF) and f) ash depends of temperature regime at anis seed storage, in %

Conclusion

Anise is an important herb that is used in milk production and as aromatic plant flavoring alcoholic beverages (mastika) and soft drinks. The performed investigations lead down to the following conclusions:

- The storage of seed material and variability in the content of organic components depends on the temperature regime.
- Carbohydrates are reduced significantly when anise (fennel) seeds are stored at room temperature, so the best option is to store them at -18°C.
- In the case of proteins, the content decreases slightly, while crude fiber (CF) decreases by almost 2,00%.
- Seed storage at room temperature in terms of fat content indicates that will drop drastically due to their involvement in seed metabolism and cellular respiration.

- An increase in ash content means a decrease in organic matter, so storage at room temperature shows almost double the content and hence it is recommended to store the seed material in a refrigerator or freezer.
- Choosing the best storage option for anise seeds at -18°C preserves its antimicrobial activity.
- The food industry needs the conservation, cultivation and use of anise landraces in the processing industry. The people should be really satisfied that local varieties that are characteristically aromatic still “live on.”

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