Selected methods for starch content determination in plant materials

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Abstract. This manuscript presents selected analytical methods for starch content determination in plant materials. The presentation includes tools and equipment needed to apply a selected method and method accuracy in determinations of starch content and parameters. An overview of these methods may be useful while designing studies focused on starch content in materials of plant origin. The manuscript also discusses the effect of microwave radiation on selected parameters of starch granules. In addition, it presents results of an experiment aimed at determining the response of potato starch granules to microwave radiation. In that experiment, the size of potato granules was measured using an OPTICA B-510BF optical microscope and PROVIEW x64 software, 3.7.13483.20181206. The results obtained were analyzed at a significance level of α=0.05 using STATISTICA 13.3 software package.

1. Introduction
Starch is the basic storage material of plants, built of two structural components: amylose and amylopectin, whose ratio is a defining feature of individual starch species [2]. It can be synthesized in chloroplasts and amyloplasts in the form of granules, and the storage can take place in several organs, including the crumb, seed endosperm, and cotyledons. The percentage content of storage starch varies depending on the plant[1].

Table 1. Selected properties of starch depending on its origin [2]

| Origin of starch | Granule shape | Granule size [μm] | Amylose content [%] |
|------------------|---------------|-------------------|---------------------|
| Algae            | spherical     | 15                | 1                   |
| Oats             | spherical     | 25                | 27                  |
| Maize            | spherical     | 25                | 52                  |
| Barley           | spherical     | 20                | 22                  |
| Wheat            | spherical     | 30                | 28                  |
| Potatoes         | oval          | 40                | 23                  |
| Peas             | oval          | 40                | 66                  |

In cereal grains, the starch content reaches 70% of fresh weight [5]. In rice grain, buckwheat grain, and wheat grain, it accounts for ca. 75%, 68-71%, and 64-70% of fresh weight, respectively. The plant species also determines the structure, size, and shape of starch granules. Their size ranges from 0.5 to 170 μm, and their shapes and structures are highly diversified and typical of a given species [6]. The
starch granules can be spherical, oval, oblong, kidney-shaped, and polyhedral. Mostly, starch granules occur singly, but in some cases they can form larger aggregates, as in the case of rice or oat starches [7,8].

a. Methods used to determine starch content

Starch analysis involves mainly determinations of its content as well as the shape and size of its granules. Several methods can be used in this respect [1].

1.1.1 The specific gravity method

The specific gravity method allows determining the percentage content of starch using a Reimann-Parow hydrostatic balance (Figure 1). The measurement is based on the correlation between the starch content and the specific gravity of tubers, and uses the Archimedes' principle. It involves weighing the potatoes in the air and then in water. Based on the obtained measurement results, the potato density and the dry matter content are calculated. The potato starch value is obtained by subtracting the content of non-starchy components (usually approximating 5.75%) from the dry matter content. This is the basic method for distinguishing starch varieties, as well as for the assessment of the starch value of potato batches purchased for the industry [1,3].

![Reimann-Parow balance](image1.jpg)

Figure 1. Reimann-Parow balance [19]

1.1.2 Pycnometric method

Another method for starch content determination is the pycnometric method (Figure 2), which is based on density measurements. It consists in comparing the weight of the same volume of liquid of unknown density with the weight of water of the same volume at the same temperature. The accuracy of measurements in pycnometric methods can be as high as 0.00001, however their drawback is the necessity to establish a constant measurement temperature [4].
1.1.3 Sedimentation method

The sedimentation method enables separating starch into fractions of various sizes and determining their percentage content [9]. It is based on the sedimentation process. Using this method, Berski et al. calculated the percentage by weight of large (with a diameter above 30.6 \text{µm}), medium (with a diameter from 21.6 to 30.6 \text{µm}), and small granules (with a diameter less than 21.6 \text{µm}) [10]. On the other hand, based on the calculated falling times of starch granules of certain sizes, other researchers [11, 12] divided starch into three fractions: large (larger than 40 \text{µm}), medium (from 25-40 \text{µm}), and small (smaller than 25 \text{µm}).

1.1.4 Polarimetric method

The polarimetric method (Figure 3) consists in dissolving starch in diluted hydrochloric acid and, after clarifying the solution, measuring the rotation of the plane of polarized light with a polarimeter. The starch content is calculated according to the Biot formula, assuming that the specific rotation of starch dissolved in HCl is 183.7°. The polarimetric method was used to assess the starch value of edible potato cultivars in studies on the correlation between contents of starch and nitrates [13]. The tested tuber samples were characterized by quite diverse starch contents. The cultivars containing from 12.0 to 16.7% of starch were defined as low-starch, these containing from 14.0 to 18.5% as medium-starch cultivars, and finally these with starch content ranging from 19.8 to 22.1% as high-starch cultivars.
1.1.5 Microscopic method

The starch granule morphology can be examined using a microscope or a laser particle analyzer. The study of the starch granule morphology includes the analysis of their surface structure, the presence of pores and other formations on their surface. These analyses are performed via highly specialized electron microscopy, using e.g. light microscope for starch granule size measurement (Figure 4). Fortuna and Juszczyk used the Jeol JSM 5200 scanning electron microscope to study the porosity of starch granules, in correlation with the susceptibility of pores to bacterial amylase [14]. In turn, scanning and transmission microscopes were used to observe the pores of maize, sorghum, and millet starch granules, as well as to visualize the pores arranged along the latitudinal furrow of large granules of wheat and barley starch [8]. On the other hand, laser particle analysis allowed determining the fraction size of starches of various plant species, including rice, oats, maize, and potatoes. Potato starch was characterized by the largest granule diameter among the studied starch varieties [15].

Figure 3. Polarimeter [17]

Figure 4. Light microscope OPTICA B-510BF [own study]
1.2 Effect of microwave radiation on starch granules

The use of microwave heating in food processing has spurred a growing interest in recent years [20, 22]. The microwave heating technology has multiple advantages, including shorter processing time, fast heat transfer, compact equipment, and environmental friendliness. Additionally, the microwave magnetic field significantly modifies the properties of the final product [23]. The effect of microwave radiation on the starch particles and structure can lead to the modification or alteration of the functional properties of starch, including its rheological properties, solubility, swelling power, and digestibility. It is assumed that microwave radiation can modify the morphology of starch granules depending on their moisture content, origin, or source [24]. The crystalline structure of starch is modified as well, due to the rearrangement of granules and destruction of the crystalline network. The extent of changes in the functional properties of starch after microwave heating depends on many factors and conditions, such as the botanical origin, moisture content, heating temperature, heating time, and absorbed microwave energy [24, 21]. Ample studies have been conducted on the impact of microwave radiation on starch grains. Cai et al. [19] investigated rice starch with different amylose contents and determined its structural (including morphology and crystallinity) and functional properties. The results of their study showed that the morphology, granule size, and crystallinity type did not differ significantly. The starches of several rice varieties were observed with a light microscope and a scanning electron microscope. They all had a similar morphology, were polyhedral and irregular in shape with sharp angles and no pores on the edges. The mean particle sizes did not differ significantly and ranged from 3.9 to 4.5 µm. In turn, the research of Zhao et al. aimed to compare changes in the structural and physicochemical properties of wheat starch after the conventional and microwave pre-acetylation processes. Microscopic examination showed that acetylation after microwave pretreatment significantly affected the surface and internal structure of starch granules compared to the conventional acetylation. The microwave-pretreated starch granules had a higher degree of substitution, acetyl content, as well as gelatinization and pasting temperatures than the untreated starch after acetylation under the same conditions. The solubility and the swelling power changed as well. Both parameters increased following the acetylation of microwave-pretreated and untreated starch. The acetylation after microwave pre-treatment significantly changed the structural and physicochemical properties of wheat starch in comparison with a single acetylation process [25]. In other studies, wheat, maize, and waxy maize starches of medium moisture content were subjected to microwave treatment, and the effect of microwave radiation on their physicochemical properties and structure was determined. Starches were examined with the Brabender rheological method, light microscopy, X-ray diffraction, and differential scanning calorimetry. The microwave irradiation reduced the crystallinity, solubility and swelling characteristics of wheat and maize starches, as well as increased the gelatinization temperature of all tested starches. The magnitude of changes induced by the microwave treatment depended not only on the crystalline structure of starch, but also on the amylose content [26]. The authors investigated the swelling behavior of three different starches in an aqueous suspension during microwave heating, using a laser particle size analyzer. There was no difference in the swelling behavior of conventional maize starch, waxy maize starch, and cross-linked waxy maize starch in an aqueous suspension between microwave heating and conventional heating. Moreover, there were no significant differences in the maximum diameter and maximum swelling temperature of these starches between the heat treatments. The results suggest that there are no differences in the swelling power of conventional and modified maize starches between the microwave-heated and the conventionally-heated samples [27].

The aim of the study was to determine the response of starch granules to microwave radiation (frequency of 2.45 GHz, microwave generator power = 100 W), measured by a change in granule dimensions. Laboratory tests were carried out in 2019 with tubers of the medium-early, edible potato cultivar Innovator.

2. Materials and methods

Potato tubers were first weighed and then irradiated with microwaves for 10 to 600 s, following the methods described by Barba et al. [28] and Jakubowski [29]. Immediately after irradiation of the tubers, starch granule size was determined based on its surface area (Pp) and the maximal diameter (Φm) (Figure 5). Measurements were made for 15 randomly selected starch granules, using an OPTICA B-510BF...
optical microscope. Images obtained were analyzed in a PROVIEW x64 software, 3.7.13483.20181206. The analyzed dependent variables (the maximal diameter and surface area of starch granules) were referred to the unitary values of microwave radiation determined in the experiment. The results obtained were analyzed at a significance level of $\alpha=0.05$ using STATISTICA 13.3 software package.

![Figure 5. Starch granules in the analyzed microscopic specimens (a – preparation made from potato tuber exposed to microwaves, b – control sample).](image)

3. Results

The performed analysis of variance and value obtained in F-Snedecor test ($F=4.550$) demonstrated a significant effect of the unitary dose of microwave radiation in the surface area and the longest diameter of starch granules (Figures 6 and 7).

![Figure 6. Correlation between the unitary dose of microwave radiation and the surface area of potato starch granules](image)
While planning the experiment, it was a priori assumed that potato tuber exposure to the microwaves having the frequency of 2.45 GHz would cause damage to starch granules, followed by their gelatinization, irrespective of exposure time and tuber size. The research results showed, however, that the unitary irradiation dose of 8.15 [J.g\(^{-1}\)] caused no significant changes in starch granule size compared to the control non-irradiated samples. It was also observed that the unitary doses ranging from 28.21 to 182.56 [J.g\(^{-1}\)] significantly decreased both the surface area and the maximal diameter of starch granules, compared to the other experimental combinations tested. In contrast, the exposure of potato tubers to the higher doses of microwave radiation (359.28-478.46 [J.g\(^{-1}\)]) increased the size of starch granules compared to the tubers irradiated at 28.21–182.56 [J.g\(^{-1}\)] (expected effect evoked by potato tuber temperature increase). The results of the study demonstrate that the microwave radiation with a frequency of 2.45 GHz can act as an abiotic stress factor which in the mentioned range of unitary irradiation doses (28.21–182.56 [J.g\(^{-1}\)]) induces a defense reaction of cells manifested by their shrinkage. In addition, as indicated by previous studies [30-33], not all starch granules are involved in the gelatinization process at the same time because granules of various sizes gelatinize at different temperatures. In the course of the gelatinization process, the structural damage is firstly observed for the large granules, whereas smaller granules are more resistant in this respect (therefore, rather the term ‘pasting temperature range’ than the term ‘pasting temperature’ is used in the literature).

4. Conclusion
The presented selected methods for starch content determination in plant materials are commonly used and sufficiently accurate in measurements made of engineering works. The overview of literature and the performed experiment indicated that the microwave radiation might affect the properties of starch granules. It was demonstrated empirically that the microwaves modified the maximal diameter and the surface area of potato starch granules. The microwaves used in unitary doses of 28.21–182.56 [J.g\(^{-1}\)] were shown to decrease, while these used in unitary doses of 359.28-478.46 [J.g\(^{-1}\)] were shown to increase granule size.
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