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Use of ImageJ Software for Assessment of Mechanical Damage to Starch Granules

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Abstract: This study attempted to assess the influence of mechanical forces on potato, tapioca, wheat, rice, and maize starch granules. For this purpose, we used digital analysis of microscopic images of starch granules before and after starch grinding using ImageJ software. Additionally, we studied the influence of temperature on the size and shape of starch granules by drying the starches for 30 min at 60 °C. Our results indicate that mechanical forces very rarely cause damage to starch granules, such as breaking or cracking. In most cases, the action of mechanical forces results only in smoother shape of starch granules and their shrinking, linked with rising temperature. Results of this study show that ImageJ software can be successfully used to assess starch granule size and shape.

Keywords: starch; mechanical damage; image analysis software; ImageJ application

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1. Introduction

Image analysis software is used more and more often to determine the quality of produced food. The analysis enables evaluation and classification of selected characteristics of products (e.g., colour, shape, size) and to determine relations between individual characteristics [1]. Qualitative and quantitative analysis of characteristics of images can contribute important information on the examined material. Image analysis software allows fast, repeatable, and completely subjective quality assessment, so it is increasingly used in studies of food products. Since Poland's accession to the EU, more attention has been paid to food quality and the methods of food production, so there is a need to search for new, fast, and effective methods for sensory analysis of food products. Instead of a group of people (employed to perform a sensory analysis of a product), who are influenced by internal factors, such as stress and fatigue, or external factors, such as temperature and light conditions, a computer is employed which is not affected by those factors. On the basis of images of products, either their morphological characteristics (e.g., size, shape, degree of infestation by pests, degree of mechanical damage) or their content of nutrients are analysed. This method can be also used to evaluate product colour, which affects decisions about its purchase by consumers. It allows for an assessment of the intensity and brightness of product colour.

Image analysis involves processing of information. The input data are provided by the image, whereas the output data can be various (e.g., numerical or text). Image analysis can be performed with the use of hardware and software tools [2,3]. The information contained in images is analysed in two ways: qualitatively (when various types of objects are identified on the basis of images) or quantitatively. The results are numerals that reflect the selected characteristics of the evaluated structure. It must be emphasized that digital image analysis outcompetes human sight in respect of qualitative image analysis. Digital image analysis is much faster and more sensitive, as while the human eye is able to distinguish between 70 shades of grey, computers and photographic equipment can distinguish between 256 or even more shades. Moreover, computers can analyse images quantitatively, which is not possible for human sight. Digital image analysis is used for many complicated transformations and repeated many times, which also gives an advantage over human sight.

Digital image analysis is currently commonly used in various disciplines, such as medicine (e.g., for tomographic cross-sections of the body), remote sensing (e.g., for interpretation of satellite images or aerial photographs), forensic science (e.g., for comparing fingerprints), or for automated sorting of correspondence [1,4–7].

One of the possible applications of digital image analysis is an assessment of the degree or dimensions of mechanical damage of starch granules. In many plant species, starch is accumulated as a storage compound in the form of starch granules, whose shape and size depends mostly on species. The potato produces the largest starch granules. They have an oval shape and sizes from 15 to 100 µm. Under an optical microscope one can see the characteristic layered structure of potato starch granules. Tapioca starch has a granules size of 5 to 35 µm and its shape varies from oval to club-shaped with a flattened end. Corn and wheat starch granules are similar in size to tapioca starch, but the shape of wheat temple granules is similar to potato starch granules (oval or round), while corn starch granules have a characteristic polyhedron shape. The smallest granules are characterized by rice starch—their size ranges from 2 to 10 µm and their shape is determined as polygonal. Rice starch usually forms characteristic clusters [8]. Starch is widely used in the food industry (e.g., to achieve a suitable texture and consistency of a product or for production of glucose, ethanol, or a commercial preparation of starch used to stiffen textile fabrics in laundering).

Damage to cereal starch granules can be a result of the action of enzymes contained in the grain but also a result of mechanical forces during its milling. Usually during grinding process, about 10–20% of starch granules are damaged [9]. An excessive level (i.e., over 30%) is unfavourable, as it lowers the baking quality of flour. During grinding, individual starch granules are separated, and many of them are also damaged [10]. The degree of damage depends on water diffusion into pores in the seed coat during pre-milling treatment and on technological conditions of the milling process. Damaged starch granules have different properties than undamaged ones: the former swell faster, absorb more water (sometimes even over 70% of the final weight), and show a three-fold higher level of adsorption since the number of small crystal structures increases and numerous crystal structures are disturbed. Such starches are more sensitive to the action of amylolytic enzymes [11–14].

ImageJ is one of the oldest programs for digital image analysis. The first version was created in 1997 at the National Institutes of Health. A very important advantage of this program is its open-source code available under a BSD-2 license. Thanks to this, it can be implemented in other projects and image analysis systems. It can also be installed in single board computers such as Raspberry Pi, which can create small systems that continuously analyze images acquired from digital cameras. Thanks to the open-source code, for over 20 years of the program's existence there have been many plug-ins created by its users which significantly increased capabilities of digital image processing and analysis [15].

This study aimed to assess the usefulness of ImageJ software for evaluation of starch granule shape and assessment of mechanical damage of starch grains from potatoes, wheat, maize, rice, and tapioca, but also for determination of the types of mechanical damage of starch grains under the influence of mechanical forces and increased temperature.

2. Materials and Methods

Material for this study comprised various starches:

- Potato starch, manufactured by the Potato Processing Company "Zetpezet" Ltd. in Pila, Poland
- Wheat starch, manufactured by Cargill Wroclaw, Poland
- Maize starch, manufactured by Chemical Works "Bochem" in Pionki, Poland
- Rice starch, manufactured in Belgium (BENEO—Remy, BE3018 Wijgmaol, Leven, Belgium),
- Tapioca starch, manufactured in Thailand.

The starches were subjected to mechanical damage by grinding in a laboratory mill (Retsch Grindomix GM200, Haan, Germany) and in a commercial kitchen appliance (Vorwerk Thermomix TM31, Wuppertal, Germany). In the laboratory mill, the starches were ground for 3 min at 10,000 rpm, and next cooled for 5 min to avoid gelatinization under the influence of the heat generated by the milling process. The total time of starch grinding in the mill was 30 min. In the Thermomix, starches were ground at 5000 rpm for 30 min without cooling intervals. During grinding, the starch temperature always increased, so control samples were not subjected to mechanical forces but were kept at 60 °C for 30 min.

From the obtained initial material samples (potato, wheat, maize, rice, and tapioca starch), five water subsamples were observed under a light microscope (Leica DM750, Heerbrugg, Switzerland) equipped with a HD camera and connected with a computer, and next 5 digital photographs of each subsample were taken at objective lens magnification of 10× using Leica LAS EZ software. In total, each sample generated 25 digital micrographs.

To determine the degree of damage to the studied starches, we analysed the micrographs by ImageJ application. It is a public domain Java image processing program for 8-bit, 16-bit, or 32-bit colour images. The application performs most of the basic analyses associated with image processing [6,7,15–17]. Each image was first subjected to binarization and next to particle analysis. We measured the area, perimeter, Feret diameter, minimum Feret diameter, and shape coefficients, namely circularity and roundness of the objects identified by the software (i.e., starch granules). (Figure 1).

The results were subjected to statistical analysis: one-way analysis of variance (ANOVA). Homogeneous groups were distinguished by the Duncan test (α = 0.05) using STATISTICA software. (TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. http://statistica.io).

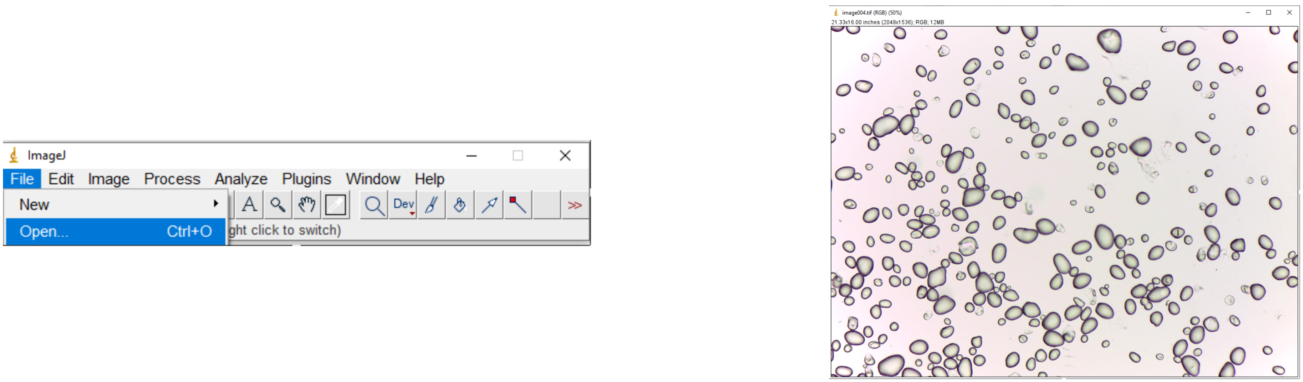

Figure 1. *Cont.*

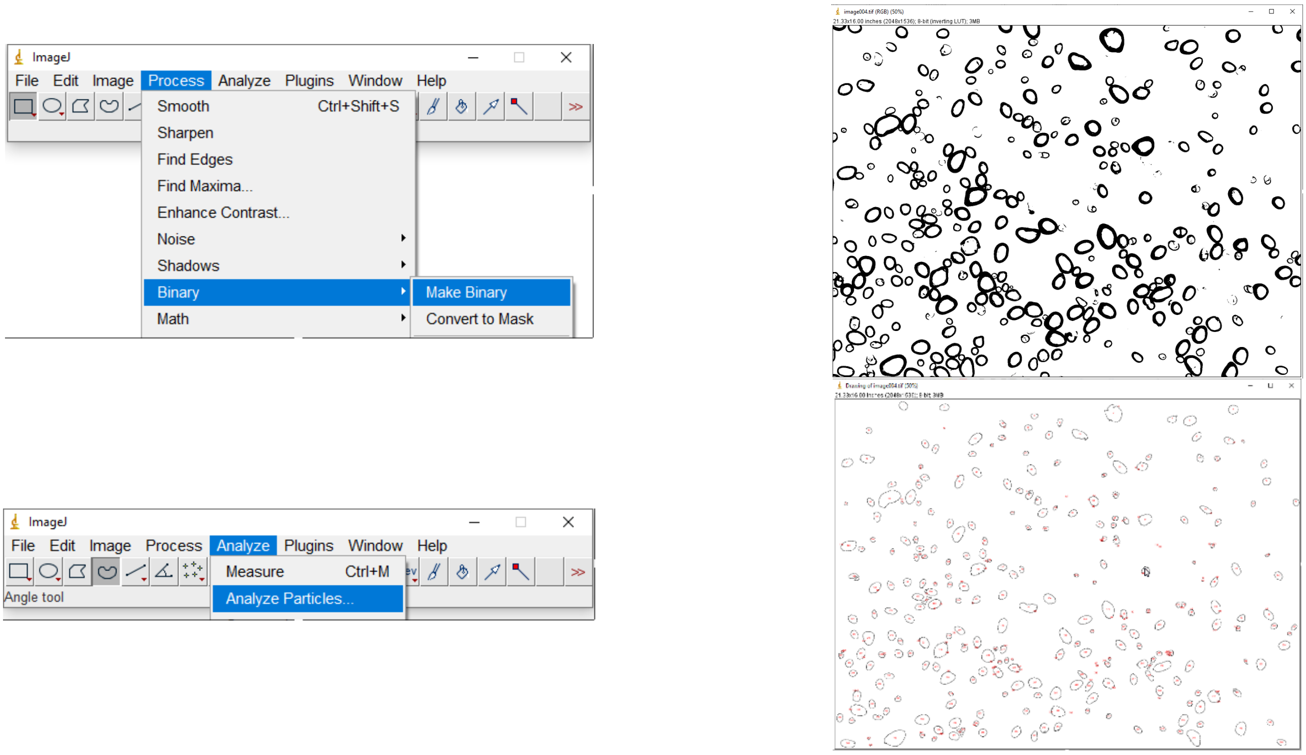

Figure 1. Diagram of the actions performed in ImageJ and their results.

3. Results and Discussion

Table 1 shows moisture and ash content of the starches used for image analysis.

Table 1. Moisture and ash content of starches used for image analysis.

	Moisture [%]	Ash Content [%/Dry Weight]
Corn starch	13.1	0.1
Wheat starch	13.2	0.2
Rice starch	3.6	0.2
Tapioka starch	12.2	0.2
Potato starch	18.3	0.4

The results of ImageJ processing of the images (Table 2), depending on plant species, show that rice starch granules were the smallest while potato starch granules were the largest. This finding is consistent with previously published data [8]. The shape of rice starch granules deviated most strongly from the circular shape (roundness = 0.72). The shape of maize starch granules was the most regular (roundness = 0.82). Potato, tapioca, and wheat starch granules were ellipsoid, and their roundness varied from 0.79 to 0.81. The differences are more strongly reflected in the circularity parameter: ranging from 0.63 for rice starch to 0.85 for potato and maize starch. Differences in Feret diameter and minimum Feret diameter indicate if the shape is more or less similar to ellipsis. The largest differences in those parameters were recorded for potato and tapioca starch, as their granules are often more elongated.

Tables 3–7 compare the results of measurements of maize, wheat, rice, tapioca, and potato starch granules before and after grinding for 30 min in a laboratory mill, Thermomix, and after drying for 30 min at 60 °C.

Table 2. Comparison of measurements of different starches before treatment.

Starch Type	Area [μm²]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Corn	77.1 ^{a} ± 21.4	32.7 ^{a} ± 10.3	11.3 ^{a} ± 1.6	8.8 ^{a} ± 1.4	0.85 ^{c} ± 0.01	0.82 ^{c} ± 0.01
Wheat	96.4 ^{b} ± 51.2	37.3 ^{b} ± 9.8	12.8 ^{b} ± 2.2	9.7 ^{b} ± 1.6	0.81 ^{b} ± 0.01	0.79 ^{b} ± 0.01
Rice	58.8 ^{c} ± 39.5	30.1 ^{c} ± 10.4	9.9 ^{c} ± 3.1	6.9 ^{c} ± 2.1	0.63 ^{a} ± 0.01	0.72 ^{a} ± 0.01
Tapioca	118.0 ^{d} ± 82.3	40.7 ^{d} ± 17.3	13.9 ^{d} ± 4.2	10.5 ^{d} ± 2.6	0.81 ^{b} ± 0.01	0.79 ^{b} ± 0.01
Potato	322.2 ^{e} ± 293.2	62.5 ^{e} ± 21.5	21.4 ^{e} ± 7.7	16.4 ^{e} ± 6.3	0.85 ^{c} ± 0.01	0.81 ^{c} ± 0.01
LSD	8.98	1.16	0.37	0.26	0.008	0.007

Values are means ± s.d. LSD = least significant difference. Different letters within columns indicate significant differences (α = 0.05).

Table 3. Comparison of measurements of corn starch granules before (control) and after grinding and drying.

Corn Starch	Area [μm²]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Control	77.1 ^{a} ± 21.4	32.7 ^{a} ± 10.3	11.3 ^{a} ± 1.6	8.8 ^{a} ± 1.4	0.85 ^{a} ± 0.01	0.82 ^{a} ± 0.01
Mill for 30 min	67.0 ^{b} ± 19.4	30.3 ^{b} ± 9.8	10.5 ^{b} ± 1.5	8.3 ^{b} ± 1.3	0.87 ^{b} ± 0.01	0.83 ^{b} ± 0.01
LSD	2.48	0.56	0.18	0.13	0.005	0.006
Thermomix for 30 min	81.0 ^{b} ± 22.5	33.8 ^{b} ± 10.4	11.6 ^{b} ± 1.7	9.2 ^{b} ± 1.5	0.85 ^{a} ± 0.01	0.83 ^{b} ± 0.01
LSD	2.75	0.61	0.20	0.14	0.005	0.006
Drying for 30 min	72.0 ^{b} ± 20.1	32.7 ^{a} ± 10.2	11.3 ^{a} ± 1.6	8.6 ^{b} ± 1.3	0.81 ^{b} ± 0.01	0.79 ^{b} ± 0.01
LSD	2.57	0.59	0.19	0.14	0.006	0.006

Values are means ± s.d. LSD, least significant difference. Different letters within columns indicate significant differences (α = 0.05).

Table 4. Comparison of measurements of wheat starch granules before (control) and after grinding and drying.

Wheat Starch	Area [μm²]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Control	96.5 ^{a} ± 51.2	37.3 ^{a} ± 9.8	12.8 ^{a} ± 2.2	9.7 ^{a} ± 1.6	0.81 ^{a} ± 0.01	0.79 ^{a} ± 0.01
Mill for 30 min	67.4 ^{b} ± 39.4	30.4 ^{b} ± 8.5	10.5 ^{b} ± 1.5	8.4 ^{b} ± 1.4	0.87 ^{b} ± 0.01	0.83 ^{b} ± 0.01
LSD	3.33	0.63	0.20	0.14	0.006	0.005
Thermomix for 30 min	78.6 ^{b} ± 41.1	33.5 ^{b} ± 8.7	11.5 ^{b} ± 1.8	9.1 ^{b} ± 1.6	0.84 ^{b} ± 0.01	0.82 ^{b} ± 0.01
LSD	3.99	0.74	0.23	0.17	0.007	0.006
Drying for 30 min	79.2 ^{b} ± 43.5	33.8 ^{b} ± 8.8	11.7 ^{b} ± 1.7	9.0 ^{b} ± 1.5	0.83 ^{b} ± 0.01	0.80 ^{b} ± 0.01
LSD	3.94	0.74	0.23	0.17	0.007	0.006

Values are means ± s.d. LSD, least significant difference. Different letters within columns indicate significant differences (α = 0.05).

In all of the analysed cases, the area of starch granules decreased significantly (both after grinding and after drying). Only in the case of potato starch was an increase in the surface of the measured starch granules found after 30 min of grinding in a laboratory mill. This result may be due to the fact that the range of the size of the potato starch granules is very large and if the granules were not broken into smaller pieces during grinding, it could be the case that in microscopic preparations after grinding large granules were more often in the field of view. Increasing the number of analysed photos would probably bring both measurements closer to each other. Some researchers reported earlier that starch granules at high temperatures shrink, losing water and changing the type of crystallinity. This is obvious in the case of starch drying, but in the case of grinding, the lower area of starch granules can be due to both their shrinking (caused by the rise in temperature during grinding) and mechanical damage to starch granules. In the case of large-sized starch granules (potato and tapioca starch), drying changes their shape coefficients only slightly (Table 6) or does not affect them (Table 7). In the case of wheat and rice starch, drying results in a slight increase in shape coefficients (Tables 4 and 5). By contrast, in maize starch granules (Table 3), drying is linked with their significant deviation from circular shape. This can be due to the less regular shape of maize starch granules, as compared to other starches.

Table 5. Comparison of measurements of rice starch granules before (control) and after grinding and drying.

Rice Starch	Area [μm^2]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Control	58.8 ^{a} ± 39.5	30.1 ^{a} ± 10.4	9.9 ^{a} ± 3.1	6.9 ^{a} ± 2.1	0.64 ^{a} ± 0.01	0.72 ^{a} ± 0.01
Mill for 30 min LSD	15.5 ^{b} ± 8.6 3.45	15.2 ^{b} ± 6.7 0.71	5.4 ^{b} ± 2.2 0.21	4.1 ^{b} ± 1.7 0.15	0.85 ^{b} ± 0.01 0.009	0.80 ^{b} ± 0.01 0.007
Thermomix for 30 min LSD	30.9 ^{b} ± 21.2 4.90	21.1 ^{b} ± 9.8 0.94	7.1 ^{b} ± 2.6 0.28	5.1 ^{b} ± 1.9 0.20	0.72 ^{b} ± 0.01 0.011	0.75 ^{b} ± 0.01 0.007
Drying for 30 min LSD	41.8 ^{b} ± 26.4 4.98	23.9 ^{b} ± 10.3 0.98	8.2 ^{b} ± 2.8 0.30	5.8 ^{b} ± 2.1 0.21	0.72 ^{b} ± 0.01 0.010	0.73 ^{b} ± 0.01 0.007

Values are means ± s.d. LSD, least significant difference. Different letters within columns indicate significant differences (α = 0.05).

Table 6. Comparison of measurements of tapioca starch granules before (control) and after grinding and drying.

Tapioca Starch	Area [μm^2]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Control	118.0 ^{a} ± 82.3	40.7 ^{a} ± 17.3	13.9 ^{a} ± 4.2	10.5 ^{a} ± 2.6	0.81 ^{a} ± 0.01	0.79 ^{a} ± 0.01
Mill for 30 min LSD	93.6 ^{b} ± 68.4 6.62	36.3 ^{b} ± 16.2 1.08	12.1 ^{b} ± 3.7 0.34	10.0 ^{b} ± 2.4 0.25	0.85 ^{b} ± 0.01 0.008	0.86 ^{b} ± 0.01 0.007
Thermomix for 30 min LSD	89.6 ^{b} ± 61.3 5.98	35.6 ^{b} ± 15.9 0.97	12.0 ^{b} ± 3.3 0.31	9.8 ^{b} ± 2.2 0.22	0.85 ^{b} ± 0.01 0.007	0.85 ^{b} ± 0.01 0.007
Drying for 30 min LSD	93.4 ^{b} ± 62.7 6.40	36.9 ^{b} ± 16.1 1.04	12.6 ^{b} ± 3.6 0.33	9.8 ^{b} ± 2.1 0.24	0.82 ^{b} ± 0.01 0.008	0.81 ^{b} ± 0.01 0.008

Values are means ± s.d. LSD, least significant difference. Different letters within columns indicate significant differences (α = 0.05).

Table 7. Comparison of measurements of potato starch granules before (control) and after grinding and drying.

Potato Starch	Area [μm^2]	Perimeter [μm]	Feret [μm]	Min Feret [μm]	Circularity	Roundness
Control	322.2 ^{a} ± 293.2	62.5 ^{a} ± 21.5	21.4 ^{a} ± 7.7	16.4 ^{a} ± 6.3	0.85 ^{a} ± 0.01	0.81 ^{a} ± 0.01
Mill for 30 min LSD	452.3 ^{b} ± 302.3 41.7	78.0 ^{b} ± 24.3 3.55	26.9 ^{b} ± 9.6 1.21	20.0 ^{b} ± 7.3 0.85	0.82 ^{b} ± 0.01 0.013	0.76 ^{b} ± 0.01 0.013
Thermomix for 30 min LSD	317.7 ^{a} ± 287.2 32.0	65.3 ^{a} ± 23.8 2.80	22.4 ^{b} ± 7.9 0.95	16.7 ^{a} ± 6.4 0.67	0.80 ^{b} ± 0.01 0.011	0.78 ^{b} ± 0.01 0.011
Drying for 30 min LSD	240.7 ^{b} ± 173.3 27.9	57.1 ^{b} ± 23.4 2.53	19.6 ^{b} ± 7.1 0.87	15.1 ^{b} ± 5.6 0.60	0.84 ^{a} ± 0.01 0.009	0.81 ^{a} ± 0.01 0.010

Values are means ± s.d. LSD, least significant difference. Different letters within columns indicate significant differences (α = 0.05).

Chong et al. destroyed the mung bean, potato, corn and waxy corn starch by grinding them in a planetary ball mill for 1 h. The greatest damage was caused by corn starch (27%), less by potato starch (13%), and the least by mung bean starch (5%) [18]. Scanning electron microscope photos showed that the outer wall of the granules is most often damaged and becomes rougher. The size of the granules is also reduced due to breaking them into smaller parts, which was confirmed by the analysis of the microscopic image. The changes in the structure of the starch granules walls are not noticeable in optical microscopy. However, image analysis showed slight, but statistically significant, changes in the circularity coefficient. Zuo et al. damaged starch granules by treating them with high-power ultrasound of up to 160 W for 30 min [19]. Also in this case, the greatest damage occurred to the structure of the walls of the starch granules. In some cases, fragments of the granules walls also chipped off. Mechanical damage to starch affects its physical properties—they lower the gelatinization temperature [10,20–22], reduce its viscosity [23,24], increase water solubility [25,26], and increase its enzymatic digestibility [27,28]

The shape coefficients after grinding show that granules of all the starches except potato starch significantly increased in circularity during grinding. This may indicate that granule shape is smoothed by their hitting one another. The analysis of changes in shape coefficients, starch granule area, and differences in Feret diameter and minimum Feret diameter allow us to conclude that grinding usually does not cause cracking or breaking of starch granules but results in delicate abrasion of their surface, which leads to their more circular shape. However, in potato starch, no such a relationship was observed. Potato starch grains are relatively large and smooth, so the abrasive effect was generally not visible after grinding for 30 min. Figure 2 shows a single damaged potato starch granule after grinding for 30 min in a laboratory mill.

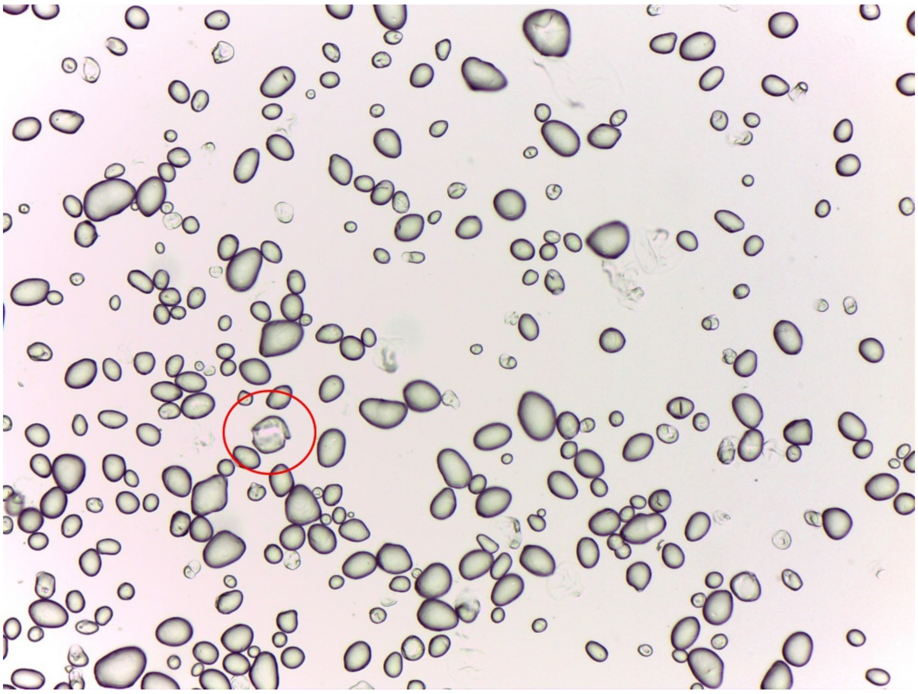

Figure 2. Damaged potato starch granule (marked with a red circle).

Much more often, we observed shrinking of starch granules under the influence of temperature and their smoother shape after grinding in a mill. The changes are more conspicuous in the starches characterized by smaller granule size and greater irregularity of shape. Changes in properties of starch subjected to mechanical forces are mostly not due to physical damage but to smoothing of the shape of starch granules and their shrinking under the influence of rising temperature during the action of quickly changing forces.

4. Conclusions

In conclusion, ImageJ software can be successfully used to assess starch granule shape and size. The software is simple and easy to use, so it can analyse large numbers of micrographs within a short time. Results of the measurements can be easily exported to spreadsheets, where they can be subjected to detailed statistical analyses. Moreover, ImageJ application is based on open source code which allows to create automated systems of digital image analysis on your own. It is possible to create one's own applications that use ImageJ system to perform image analysis and use the results obtained in this way for other purposes. The results of the work indicate the possibility of constructing cheap, easy to use, and fast automated systems for starch quality control, in particular, the degree of starch granules damage at different stages of processing.

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