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RESEARCH ARTICLE

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Physiochemical and Rheological Properties of Starch from Different Botanical Sources

Dlir Amin Sabir

Department of Food Sciences and Quality Control, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimanyah, IRAQ.

*Corresponding Author: dlir.sabir@univsul.edu.iq.

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ABSTRACT

In this study, starches derived from a wide variety of plants (including wheat, white barley, black barley, black maize, white maze, yellow maze, rice, oat, millet, rye, triticale, and potato) were tested for their chemical, physical, and rheological properties. The compositional and architectural features of starch granules affect the accessibility of enzymes to the interior of the granule, which in turn affects the hydrolysis of native starch. To learn how cultivar specific variation regulated hydrolysis using amylases, we employed starches derived from various plant sources. Chemical methods were used to determine the starch's composition, while Amylograph and digital microscopy were used to measure the starch granules and learn more about their structure. Starches derived from several plants have varying chemical compositions, with values ranging from 21.67 to 31.33%, 60.33 to 86.00%, 0.87 to 9.23%, 0.13 to 1.33%, 0.27to 1.20% ,0.53to1.20% and 11.90 to13.33% respectively in term of amylose, α-glucan, β-glucan, protein, lipid, ash and moisture. According to the rheological analysis results, which ranged from 55.67 to 86.00 ºC, 10.00 to 25.33 g/g, 2.67 to 24.67 %, and 8.33 to 55.33 (μm) for Gelatinisation Temperature, Solubility, Swelling Power and Average Diameter Size. While the Hydrolysis rate under different temperature degrees (50ºC, 70ºC, and 90ºC) ranged between 1.83-29.67%, 54.00-72.33%, and 64.33-85.33%, respectively.

***Keywords*:** Starches, granule size, β-glucan, swelling, solubility.

INTRODUCTION

Most starches come from cereals and have a wide range of compositional, morphological, thermal, and rheological characteristics. The starch found in plants is the most vital organic substance on Earth. The majority of the calories consumed by humans come from starch. The most common sources of starch include cereal grains, tubers, and legumes. They contribute to the energy and nutritional requirements of many people in developing countries and constitute an important source of employment and income [1].

Starch, the most abundant carbohydrate in plants, is found in granules inside plant cells. The amylopectin fraction of starch is responsible for its crystallinity, while the amorphous fraction primarily represents amylose [2]. It has been noted that the starches of different species have slightly varied arrangements of amylose and amylopectin within the granules.

The osmotic pressure of a cell is barely affected by the insoluble nature of native starch granules in cold water. Depending on their botanical origins, starch granules can range from 1 to 100 (μm) in size [3]. Compared to other sources of starch, the granules found in cereals are extremely tiny. The physiochemical properties of starch and the methods used to refine it are affected by the granule size. The term granule size describes the typical starch granule size. Amylose and amylopectin fractions have distinct molecular structures that shift depending on granule size. The gelatinization capabilities of starch vary with the size of its granules. Starch granule size has been found to affect amylose-lipid interactions, which are a property of starch [4]. Solubility, swelling power, and chemical makeup, especially amylose and lipid content, relate to starch granule size [5].

This research was important because it shed light on the functioning and structural parts of starches, such as amylose and amylopectin, β-glucan, swelling power, and water solubility index, and their effect on the end-use products of starches from different botanical sources.

Materials and Methods

Cereal starches

Twelve different cereal grains and tubers from different botanical sources were selected from Sulaimani's local market (wheat, white barley, black barley, black maize, white maze, yellow maze, rice, oat, millet, rye, triticale, and potato) for this research.

Starch Extraction

In a cold solution of 2.5g sodium metabisulphite and1g of sodium chloride dissolved in 100 ml of distilled water concentrate (1% of both salts), the starch was washed, peeled, and then liquidized. The samples were left out overnight at room temperature. The starch and non-starch components were separated using a 150 m sieve. The supernatant was thrown away after centrifuging the filtered starch liquor (1500 g for 5 minutes). Impurities were separated of the crude starches by centrifuging them through a solution of 80% (w/v) caesium chloride (3000g for 20 minutes). After being centrifuged (1500 g for 5 minutes) to remove excess acetone, the purified starches were spread out on glass plates to dry in the air [6].

Particle Sizing

Starch granule dimensions were determined by microscopic observation Images, from separate areas each with approximately 100 starch granules, were randomly recorded using a Motic D M 111 Digital Microscope Macintosh and Windows Compliant with a 1000X oil microscopic objective from Speed Fair Co, LTD, Hong Kong. After 3 hours of drying at room temperature, the starch was suspended in 95% ethanol, and the samples were coated using slides (75 mm x 25 mm) [7].

Chemical and Enzyme Analysis of Starch

Moisture Content

Test samples of starch (100 mg in triplicate) were weighed and then dried in an air-forced oven at 130 degrees for one hour. After 30 minutes of cooling in desiccators, the samples were reweighed. The percentage of sample weight loss [8] was used to determine the moisture content.

Ash Content

The percentage of sample weight lost was used to determine the ash content of cereal starches [9].

α-glucan Content

Enzymatic hydrolysis of starch yields glucose, which was used to calculate the starch (total glucan) content using the method [10]. Starch samples (100 mg) were dissolved in 85°C bacterial amylase and entirely converted to glucose in 60°C amyloglucosidase. Then, glucose was measured colorimetrically with glucose-oxidase peroxidase chromogen (GOP), and the amount of glucose was converted to anhydrous starch using a factor of 0.9.

β-glucan Content

β-glucan content was determined according to [11]. The enzyme kit contains exo-1,3-β-glucanase, β-glucosidase, amyloglucosidase and invertase; glucose determination reagent (glucose oxidase peroxidase, and 4-aminoantipyrine), and glucose standard solution. Measurement of total glucan content was conducted by hydrolyzing the shiitake samples with 37% hydrochloric acid (v/v) for 45 min at 30℃ followed by an additional 2 hr at 100℃. After neutralization with 2M potassium hydroxide, glucose hydrolysis was performed using a mixture of exo-1,3-β-glucanase and β-glucosidase in sodium acetate buffer (pH 5.0) for 1 hr at 40℃. The absorbance of the resulting color complex was measured at 510 nm using a spectrophotometer. The β-glucan content was calculated by difference by subtracting the α-glucan content from the total glucan content.

β-glucan (% w/w) = Total glucan (% w/w) - α-glucan (% w/w)

Amylose Content

The amount of amylose was calculated calorimetrically using the formula [12]. From the blue result (defined as the absorbance of 10 mg of anhydrous starch in 100 ml of diluted I2-KI solution at 635 nm), the total amylose content was determined using the following formula.

Amylose (%) = (28.414 × Blue Value) - 6.218

Protein Content

Samples of starch were analysed for their nitrogen content, and the results were found in [13]. From which the protein percentage was derived:

Protein (%) = Nitrogen (%) ×6.25

Fat Content

The starch lipid content was calculated using the formula [14]. Hydrochloric acid (HCl) is used to acid hydrolyse the sample, and then the extracted lipid components are extracted with mixed ethers to estimate the crude fat content. The ethers are removed, and the lipid residue is heated to a constant weight of 100 degrees Celsius. The residue is measured using crude fat percentage.

Physical Analysis of Different Selected Starches

Determination of swelling power and water solubility index:

Water solubility index (WSI) and swelling power (SP) were measured in the g/g (95ºC) temperature range using the method of [15].

Rheological and Thermal Properties

Amylograph performed rheological tests by mixing 60 g of flour with 440 mL of distilled water according to [16].

Result and Discussion

Data in table one indicates that there were significant differences between chemical composition of cereals from different botanical sources wheat, white barley, black barley, black baize, white maze, yellow maze, rice, oat, millet, rye, triticale, and potato in term of amylose, α-glucan, β-glucan, protein, lipid, ash and moisture content the highest values obtained for rye, rice, white barley, oat, black maize, black Maize, potato (31.33, 86.00, 9.23, 0.70, 0.67. 1.20,1.20, and 13.33) respectively. It has been reported that the low hydrolysis rate observed for starch containing high amylose contents might be related to retro gradation of amylose a process during hydrogen bonds are formed between amylose molecules and amylopectin. The retrograded amylose is very stable to heat (up to 120^{0}C) [17].

Table 1. Proximate chemical composition of starches from different botanical sources.

Starch Source	Amylose (%db.)	α-Glucan%	β-Glucan%	Protein (%db.)	Lipid (%db.)	Ash (%db.)	Moisture %
Wheat	29.00	76.33	4.33	0.13	0.57	0.77	12.60
White Barley	30.00	75.67	9.23	0.37	0.30	0.73	12.47
Black Barley	29.67	76.00	8.87	0.33	0.37	0.83	12.17
Black Maize	21.67	84.00	7.33	0.30	1.20	1.20	12.43
White maize	22.00	84.33	5.00	0.47	0.33	0.97	12.43
Yellow Maize	21.67	80.33	5.33	0.63	0.53	1.00	12.20
Rice	21.67	86.00	0.87	0.37	0.67	0.87	11.90
Oat	25.00	64.00	4.33	1.33	0.27	0.77	12.07
Millet	26.33	60.33	8.67	0.30	0.30	0.80	12.33
Rye	31.33	63.33	3.33	0.70	0.33	0.73	12.00
Triticale	22.33	62.00	2.83	0.30	0.50	0.60	12.70
Potato	27.00	77.33	4.13	0.20	0.33	0.53	13.33
$LSD_{0.01}$	6.02	6.64	1.23	0.60	0.50	n.s	n.s
Grand Mean	25.64	74.14	5.36	0.45	0.48	0.82	12.39
Lower limit 99%	22.29	65.77	2.99	0.16	0.24	0.66	12.04
Upper limit 99%	28.98	82.51	7.72	0.74	0.71	0.98	12.73

Starch surface protein has been reported to reduce the hydrolysis of starch granules by enzyme [18]. Amylose has a polymerization degree poly (DP) between 300 and 5000. However, it is now accepted that some compounds exhibit weak branching via - (1-6) connections [19]. However, the number of branch points is insufficient to change the amorphous nature of amylose in typical starch granules [20]. The addition of lipids to amylose can raise the temperatures required for starch gelatinization, change the paste's textural and viscosity characteristics, and impede retro gradation. Lipids and proteins are known to be bound to starch granules, with their presence on the surface and within the granules depending on the method of extraction used. Proteins and lipids on the starch's outer layer are distinct from those on the granule's interior. The presence of internal lipids is one of the key properties of cereal starches, whereas tuber and root starches contain less lipid and protein [21].

Generally the grand mean for all cereals from different botanical sources like wheat, white barley, black barley, black maize, white maze, yellow maze, rice, oat, millet, rye, triticale, and potato in term of amylose, α-glucan ,β-glucan ,protein , lipid , ash and moisture were (25.64, 74.14, 5.36, 0.45, 0.48 ,0.82 and 12.39%) respectively while the confidence Internal ranged between (22.29 -28.98, 65.77-82.51, 2.99-7.72, 0.16-0.74, 0.24-0.71, 0.66-0.98 and 12.04-12.73 %) respectively . That mean the chemical composition percentages for overall all of cereals from different botanical sources mentioned above are not less than 22.29 65.77 2.99 0.16 0.24 0.66 12.04% and not more than 28.98, 82.51, 7.72, 0.74, 0.71, 0.98, 12.73 respectively.

Table 2. Physical analysis of starches from different botanical sources.

Starch Source	Gelatinization Temperature (C^0)	Swelling Power g/g (95^{0}C)	Solubility% (95^{0}C)	Average Diameter Size (µm)
Wheat	63.33	21.67	2.67	21.17
White Barley	61.67	22.00	15.33	13.83
Black Barley	64.00	25.33	20.33	11.33
Black Maize	80.33	18.00	4.00	15.50
White maize	81.33	24.67	5.00	13.67
Yellow maize	80.67	21.33	3.67	15.83
Rice	86.00	10.00	4.33	55.33
Oat	55.67	10.00	11.33	45.00
Millet	73.00	14.67	13.33	8.33
Rye	56.67	13.33	4.33	24.17
Triticale	59.67	19.33	3.67	26.67
Potato	80.00	14.00	24.67	46.67
$LSD_{0.01}$	6.31	5.12	3.25	5.49
Grand Mean	70.19	17.86	9.39	24.79

Table 2. Physical analysis of starches from different botanical sources.

Starch Source	Gelatinization Temperature (C^0)	Swelling Power g/g (95^0C)	Solubility% (95^0C)	Average Diameter Size (µm)
Lower limit 99%	60.25	13.05	2.67	10.75
Upper limit 99%	80.14	22.68	16.11	38.84

The data in table 2 indicates significant differences between the physical analysis of starches from different botanical sources regarding gelatinization temperature, swelling power, solubility and average diameter size. Gelatinization is an important property of starch molecules for various processing operations. In most industrial applications starches are heated in aqueous dispersions starch in its native form has semi crystalline order in which the starch molecules are aligned and hydrogen bonded to each other, excluding water and resisting enzymatic activity. It is widely accepted that the starch granule's crystalline order is a fundamental determinant of many of its functional features, and that this order must be broken for efficient enzymatic hydrolysis to occur [22].

It has been reported that the amorphous amylose in normal potato starches reduces the relative amount of crystalline material in the granules [21], a phenomenon that is observed across the gelatinization temperatures of cereal starches such as wheat, white barley, black barley, black maize, white maze, yellow maze, rice, millet, rye, triticale, and potato. These numbers are similar to the numbers provided by [22], which state that the gelatinization temperatures of starches are affected by factors such as granule form, the proportion of large to small granules, and the presence of phosphate esters. Retrogradation describes the re-crystallisation tendency of gelatinised starches upon cooling and storage. Scientists and technologists in the food industry are very interested in these phenomena because of their significant impact on the taste, texture, and longevity of starch-based foods.

For swelling power, black barley had the most significant values (25.33g/g), while rice and oat had the lowest results (10.00g/g) and solubility had the highest values (20.33%) and the lowest was for triticale (3.67%). Starch and water interact, and this may be seen in the water solubility and swelling power values. According to [23], these numbers corroborate claims. The ratio of amylose to amylopectin and the length of the starch chains all have a role in the swelling potential of a starch. Starch swelling is facilitated by amylopectin's ability to absorb and store water due to its highly branched structure [24]. As the starch is heated, the long chain amylose is released from the granules and spreads across the crystalline region. In contrast, the short chain amylopectin fills in the granules' internal network, increasing the starch's stability and swelling capacity [25]. According to the average diameter size of the native starches was observed from the highest values for Potato (46.67µm). In comparison, the lowest results were obtained for Millet (8.33 µm) starch has been reported to have relatively broad granule size ranging from 5 to 100µm, diameter of 23-30µm, comparable to the data reported by [26]. Native starch granules are insoluble in cold water and exert a minimal effect on the osmotic pressure of the cell. Starch granules vary in their granular shape, dimensions and size distribution according to their botanical origins [27]. Cereal starch granules are particularly small compared to other starch granules like tubers and root starches. The granule size influences the physico-chemical characteristics of starch, and the procedures employed for starch refining crystalline structure and particle size are the most important factors regulating starch hydrolysis by α-amylase. The physical properties of starch structure are responsible for specific uses in the food and manufacturing industries.

Generally, the Grand mean for all cereals from different botanical sources like wheat, white barley, black barley, black maize, white maze, yellow maze, rice, oat, millet, rye, triticale, and potato in term of amylose, α-glucan, β-glucan, protein, lipid, ash and moisture content were 70.19, 17.86,9.39 ,24.79 respectively. At the same time, the confidence Internal ranged between 60.25-80.14 ,13.05- 22.68, 2.67-16.11,10.75-38.84 respectively. That mean the physical properties of starch for overall all of cereals from different botanical sources.

Table 3. Hydrolysis rate (%) of pre-heated native starch starches from different botanical sources with fungal α amylases.

Starch Source	Hydrolysis rate (%)		
	Pre-heating incubation temperatures (50ºC)	Pre-heating incubation temperatures (70ºC)	Pre-heating incubation temperatures (90ºC)
Wheat	6.00	67.00	85.33
White Barley	2.80	64.67	73.67
Black Barley	3.40	60.00	70.00
Black Maize	15.00	60.67	72.67
White maize	22.33	62.33	80.67
Yellow maize	23.00	64.67	81.00
Rice	29.67	72.33	80.67
Oat	4.67	64.67	73.67
Millet	13.33	54.00	64.33
Rye	2.33	54.00	73.00
Triticale	4.67	63.00	74.00
Potato	1.83	65.67	77.00

$LSD_{0.01}$	3.99	5.80	9.31
Grand Mean	10.75	62.75	75.50
Lower limit 99%	2.07	58.11	70.37
Upper limit 99%	19.43	67.39	80.63

above are not less than 60.25, 13.05, 2.67, 10.75 and not more than80.14, 22.68, 16.11, 38.84 respectively. Data in table three indicates that there were significant differences between physical analysis of starches from different botanical sources in term of the hydrolysis rate under different temperatures degrees like pre-heating incubation temperatures (50ºC), (70ºC) and (90ºC). the highest hydrolysis rate obtained by rice, potato and wheat 29.67, 65.67 and 85.33 respectively. The susceptibility of starch to α-glucan enzymatic hydrolysis depends on the botanical origin and treatment conditions. Tuber starches are more susceptible than legume or cereal starches towards heat–moisture treatment, among which the potato starches show a relatively high increase in α-amylase hydrolysis upon heat–moisture treatment compared to the other root and tuber starches [28]. Heat-moisture treatment of different root and tuber starches, increase in hydrolysis could be attributed to the interactions involving amylose chains. Amylose form b-type crystals for different melting temperatures [29].

Conclusion

Plant-based starches have different physicochemical, rheological, thermal, and retro gradation characteristics. The food business has a high demand for starches that possess unique functional qualities. Starches with desirable functional qualities significantly contribute to enhancing product quality. Chemical, physical, and rheological characteristics of 12 plant-based starches were studied. The results showed that:

- Wide range of amylose ratio was observed among the starches.
- Gelatinization temperature reflects the percentage of large and small granules.
- The granular size appears to determine the accessibility of the granules to enzymatic hydrolysis.
- Hydrolysis of starches by α-amylase showed different patterns.

Suggestions for future work. This work has focused on the amylolysis of native starches to understand how the composition and structure of the starches control their digestion and health effects. An in vivo study, probably using rats, would be highly desirable to correlate the data generated in this study with a proper digestive process.

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الخصائص الفيزيائية والكيميائية والريولوجية للنشا من مصادر نباتية مختلفة

دلير أمين صابر

اقسم علوم الأغذية ومراقبة الجودة، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، العراق.

الخلاصة

في هذه الدراسة، تم اختبار النشويات المشتقة من مجموعة متنوعة من النباتات يتضمن (القمح والشعير الأبيض والشعير الأسود والذرة السوداء والذرة البيضاء والذرة الصفراء والأرز والشوفان والدخن والشيلم والتريتيكال والبطاطس) لخصائصها الكيميائية والفيزيائية والريولوجية. تؤثر السمات التركيبية والشكلية لحبيبات النشا على إمكانية الوصول الإنزيمات إلى داخل الحبيبات، مما يؤثر بدوره على التحلل المائي للنشا الأصلي بواسطة الأميليز. لمعرفة كيفية دخول الاختلاف الخاص بالصنف التحلل المائي باستخدام الأميليز, استخدمنا النشويات المشتقة من مجموعة متنوعة من المصادر النباتية. تم استخدام الطرق الكيميائية لتحديد تكوين النشاء، بينما تم استخدام الأميلوغراف والفحص المجهري الرقمي لقياس حبيبات النشا ومعرفة المزيد عن بنيتها. النشويات المشتقة من عدة نباتات لها تركيبات كيميائية متفاوتة، بقيم تتراوح من 21.67 إلى 31.33 ٪، 60.33 إلى 86.00 ٪، 0.87 إلى 9.23 ٪، 0.13 إلى 1.33 ٪، 0.27 إلى 1.20 ٪، 0.53 إلى 1.20 و 11.90 إلى 13.33 ٪ على التوالي للأميلوز، الفاكلوكان ،بيتا كلوكان، بروتين، دهون رماد ورطوبة. وفقا لنتائج التحليل الريولوجية تراوحت القيم من 55.67 الى 86.00 درجة مئوية, 10.00الى 25.33جم\جم,2.67الى24.67ملي مايكرو ن لكل من درجة حرارة الجلتنة, الذوبانية, قوة الانتفاخ ومعدل حجم القطر. بينما معدل التحلل المائي تحت درجات حرارية مختلفة 50,70و90 درجة مئوية تراوحت بين 1.83الى29.67%, 54.00الى72.33% و64.33الى85.33% على التوالي.

<u>الكلمات المفتاحية</u>: النشويات، حجم الحبيبات، بيتا كلوكان، الانتفاخ، الذوبانية .