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EFFECT OF ALKALINE EXTRACTION ON THE FUNCTIONAL AND PHYSICOCHEMICAL PROPERTIES OF STARCH EXTRACTED FROM RICE, MILLET, AND OATS.

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ABSTRACT

aimed to investigate the effect of alkaline extraction, using different concentration sodium hydroxide (NaOH) solution (0.2, 0.4, 0.5, 0.6)% on the physicochemical and functional properties and final yield of rice, millet and oat starch. The results showed that the yield of starch from rice flour ranged between (50 - 68.18)% and that the highest yield of starch was achieved using alkaline solution (NaOH) 0.4%, while for millet and oat the yield of starch ranged between (47.46 - 54)% and (48.3 - 62.66)% respectively, and the highest yield was recorded with (0.2)% (NaOH) for both crops. The percentage of amylose was high in the starch sample which was extracted using a low concentration of alkaline solution. The percentages of amylose were inversely related to functional properties (swelling capacity and solubility) which increased when the percentage of amylose in the obtained starch decreased. The highest values of swelling capacity were (10.34)%, (8.11)% and (8.97)% for rice, millet , and oat starch samples.

Keywords: **amylose, amylopectin, chemo physical characteristics.**

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INTRODUCTION

Starch is one of the most abundant polysaccharides in nature, along with chitin and cellulose. It has wide-ranging food applications due to its functional properties, including moisture retention, gelatinization, and viscosity, in addition to its widespread consumption as an important source of energy. Starch is found in the form of granules in the endosperm of cereal crops and in legume seeds and tubers, and is the main carbohydrate store in green plants. Starch granules are either oval or spherical, and their shape largely depends on the plant source. Starch granules generally range in size from 3 to 25 µm. There are two types of granules in cereal starch: the first type, called type A granules, are smaller (3–6 µm), while the second type, known as type B granules, are larger (10–25 µm). The two types differ in their content of secondary components, such as protein, fat, and ash, as well as in their chemical and physical properties, including gelatinization and pasting temperature. Starch is composed of two homopolymers of glucose units: amylose and amylopectin. Amylose is a linear polymer consisting of glucose units linked by α (1-4) glycosidic bonds. Amylopectin is a branched polymer composed of glucose units connected by α (1-4) glycosidic bonds, with branches linked by α (1-6) bonds. In 'normal' starches, the amylose content is about 25-30%, while the amylopectin content is around 70-75%. When amylopectin content is high, between 98-99%, these starches are referred to as 'waxy' starches (Agama-Acevedo *et al*., 2019; Wang *et al*., 2020). Starch granules contain different layers of crystalline and amorphous regions formed by amylose and amylopectin. The physical and chemical properties of starch are linked to the amylose/amylopectin ratio. Various methods and chemicals are used to extract starch from grains and dissolve proteins that form a matrix around starch

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granules along with other cellular structures. The starch extraction process is primarily influenced by the solubility of protein and the level of starch-protein interactions. Additionally, starch extraction is affected by the properties of the starch granules, such as morphological features including shape and granule diameter (El Halal *et al*., 2019). Rice (*Oryza sativa*) is one of the world's most important cereal crops, ranking second in importance after wheat. Rice grains contain approximately 5.96% protein, 1.24% fat, 7.07% fiber, 0.39% ash, and 80.14% starch (Shakri *et al*., 2021). Rice and oats primarily contain glutelin and globulin proteins, respectively. Various methods exist for isolating starch from rice, including the use of alkaline solvents, surfactants, or proteolytic enzymes to remove proteins from rice flour (El Halal *et al*., 2019). Oats (*Avena sativa L.*) is an important and versatile crop, primarily used for food and feed. Oat grains contain approximately 17.1% protein, 6.4% fat, 11.3% fiber, 3.2% ash, and 52.8% starch. Millet (*Panicum miliaceum*) has various health benefits, and its increased use in food applications is due to its gluten-free nature. Millet contains about 4% fat and 8% protein and is a rich source of minerals and vitamins, with a relatively high carbohydrate content (Al-Mhyawi, 2023). Different extraction methods have different properties and efficiencies in terms of reducing protein, fat and ash content. There are three main methods for efficiently separating proteins from starch: aqueous extraction, alkaline extraction, and the use of protease enzymes. According to previous studies, the highest yield of grain starch was obtained through the action of protease enzymes, followed by alkaline extraction using sodium hydroxide or calcium hydroxide. Although the enzymatic method using protease appears more efficient than alkaline treatment in removing proteins, the alkaline method is more effective at removing fats, likely due to partial saponification and subsequent removal of fatty acids (El Halal *et al*., 2019). Given the nutritional importance of cereal starch, the current study aimed to optimize the alkaline extraction (using NaOH) condition for starch from three different crops (rice, millet, oat) and the impact of that on the morphological and functional properties.

MATERIALS AND METHODS

Materials: Jasmine variety rice grains (harvest 2023) were obtained from the Ministry of Agriculture/Directorate of Agricultural Research. Oat grains and millet grains were purchased from local markets, and the grains were inspected and certified by Ministry of Agriculture / Directorate of Seed Testing and Certification.

Rice grains grinding: The blanched rice grains were ground using a household grinder after adjusting the moisture content to 14% according to the method followed by (Jassim, 2020) using distilled water (applying the hydration equation). The grains were stored for 24 hours in a sealed glass container with shaking every two hours.

The amount of water to be added (cm^3) = $\frac{100-\text{Moisture of sample}}{100-\text{ Desired moisture}} - 1 \times \text{Weight of sample}$

The resulting powder was passed through a standard 300 µm mesh sieve and then stored in polyethylene bags in a freezer at -18°C until further tests were conducted.

Grinding millet and oat grains: The previously cleaned millet and oat grains were ground after conducting moisture testing using a rapid moisture tester, with two replicates. The tempering process was then carried out according to the methods of (Jassim, 2020) to adjust the moisture content of the grain samples to 10% using distilled water in two stages over 48 hours at room temperature. The grains were placed in sealed containers and shaken regularly every two hours, with the amount of water added calculated as per the equation mentioned above. The technical grinding process for the tempered millet and oat samples was conducted to obtain flour with a 71% extraction rate using the Laboratory Mill available at the College of Agricultural Engineering Sciences / Food Science Department / Grain Processing Laboratory, equipped by German company Brabender (Quaternary Brabender Mill).

Sieving process: For purification, a Circular Vibration Sieve equipped with standard sieves arranged in sequence (No. 70, No. 60, No. 50,

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No. 16) was used to standardize the particle sizes of all types of samples under study. The bran was separated, and the endosperm lumps that did not pass through the No. 70 sieve were re-ground using a household grinder. The resulting flour was then re-sieved through the No. 70 sieve.

The extraction rate was determined using the following equation:

$$\text{Extraction percentage}=\frac{\text{Weight of Flour}}{\text{Weight of Bran}+\text{Weight of Flour}}\times 100$$

Extraction of grains starch

Starch was extracted from the grain sources under study using the alkaline extraction method described by (Verma *et al.*, 2018) with some modifications. The rice (*Oryza sativa*) varieties for the treatments (TR6, TR5, TR4, TR2) were used, as well as millet (*Panicum miliaceum*) for the treatments (TM6, TM5, TM4, TM2), and oats (*Avena sativa*) for the treatments (TO6, TO5, TO4, TO2). The flour was suspended in an alkaline solution at a ratio of 1:6 (flour: alkaline) with concentrations of (0.6, 0.5, 0.4, and 0.2) % equal to (0.15, 0.125, 0.1, and 0.05) molar respectively for rice, millet, and oats, respectively.

Estimation of amylose and amylopectin content: The percentages of amylose and amylopectin were estimated using the method described by (Bangar & Siroha, 2022). The colorimetric method was used to determine the amylose content using iodine blue dye. Amylopectin content was estimated using the following equation:

$$\text{Amylopectin percentage}=100-\text{Amylose percentage}$$

Chemical composition of flour and starch grains: Moisture content was determined based on the standard method of the American Association of Cereal Chemists (A.A.C.C International), method number 10.01 – 39, as followed by (Al-Mhyawi, 2023). Ash content was determined according to method number 80.01.01, and protein content was determined using method number 46-13.01, as followed (Mahajan *et al.*, 2021), 11th edition, 2010). Oil content was measured using the method followed by (Al-Mhyawi, 2023). Total fiber content was determined according to the method described in A.A.C.C (2016) and cited by (Bangar & Siroha, 2022). Carbohydrate content was calculated as described by (Jassim, 2020) by subtracting the sum of the percentages of moisture, protein, fiber, and ash from 100.

Estimation of starch functional properties;

Solubility and swelling capacity; the starch solubility and swelling capacity of samples were determined according to (Al-Muhyawi, 2023).

Water holding capacity (WHC); The water holding capacity of the rice, millet, and oat samples was determined according to (Al-Mhyawi & Nasser, 2023).

Oil holding capacity (OHC); The oil holding capacity of the starch samples was determined according (Al-Mhyawi & Nasser, 2023).

Estimation of pH Value: The pH value was estimated using the method described by (Al-Muhyawi, 2023) with some modifications.

Statistical analysis; The Statistical Analysis System (Statistical Analysis System - SAS 2018) was used to analyze data to study the effect of different treatments on studied treatments according to a Completely Randomized Design (CRD). The significant differences among the means were compared using the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Extraction rate: (Table 1) shows the starch extraction rate for the studied grains. The results indicated that the highest extraction rate of rice variety was recorded in treatment (TR4), with an extraction rate of (68.18%), which is much higher than treatments (TR2, TR5, TR6), with extraction rates of (56.93, 54.00) %, and (50.00%), respectively. The extraction rate obtained for treatment (TR4) was higher than (66.5%) reported by (Arns *et al.*, 2015). The differences between treatments were statistically significant. The starch yield from millet for treatment (TM2) was (54%), higher than treatments TM6, TM5, and TM4, which were (50.18 %, 47.46%, and 48.1%), respectively. The result for treatment (TM2) is similar to that (54.1%) reported by (Nakorn *et al.*, 2009), while the result for treatment (TM6) was close to that reported (52.82%) by (Wijesinghe & Gunathilake, 2020). The differences among the treatments were

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statistically significant. Regarding the oat starch yield for treatment (TO2) was (62.66%), which is higher than the yields for treatments TO6, TO5, and TO4, which were (50%, 48.3%, and 50.75%), respectively. The starch yield for treatment (TO2) was higher than those reported by (Kumar *et al*., 2018), which was (47 ± 4%). The differences among the treatments were statistically significant. Wijesinghe & Gunathilake (2020) indicated that the starch yields for rice and millet were (64.1% and 52.8%), respectively. Bindar & Efan (2013) stated that the optimal concentration of the alkaline solution (NaOH) for extracting rice starch was (0.4%), and that gelatinization occurs at higher concentrations of NaOH, hindering the extraction process, which is consistent with the findings of this study. The differences between the current study's results and previous research may be due to several factors, such as starch content in the grains, grain quality, pre-treatment, production methods, and separation techniques, among others. As for the variation between the starch yield of rice flour, millet and oats, El Halal, *et al* (2019) stated that the extraction of starch is mainly affected by the solubility of protein and the level of protein-starch interactions, as protein solubility reflects the balance between protein-solvent interactions (hydrophilic) and protein-protein interactions (hydrophobic), and starch isolation is affected by the properties of starch granules and morphological features including the shape and diameter of the granule.

Table 1. The yield%, of amylose, amylopectin, and amylose/amylopectin ratio of starch sample under study

	Treatment	Yield%	Amylose /amylopectin ratio	Amylopectin ratio%	Amylose ratio%
Rice	TR6	50^{c}	23.40^{a}	76.60^{a}	0.30^{a}
	TR5	54^{bc}	24.40^{a}	75.40^{a}	0.33^{a}
	TR4	68.18^{a}	24.90^{a}	75.10^{a}	0.33^{a}
	TR2	56.93^{a}	26.23^{a}	73.77^{a}	0.35^{a}
	L.S.D	* 5.49	NS	NS	NS
Millet	TM6	50.18^{b}	32.33^{b}	67.67^{a}	0.48^{a}
	TM5	47.46^{b}	33.31^{b}	66.69^{a}	0.50^{a}
	TM4	48.10^{b}	34.76^{b}	65.24^{a}	0.53^{a}
	TM2	54^{a}	37.93^{a}	62.07^{a}	$^{a}0.61$
	L.S.D	* 3.79	* 3.067	* 4.05	* 0.109
oat	TO6	50^{b}	30.74^{b}	69.68^{a}	0.43^{a}
	TO5	48.30^{b}	30.22^{b}	69.26^{a}	0.44^{a}
	TO4	50.75^{b}	31.22^{b}	68.78^{a}	0.45^{a}
	TO2	62.66^{a}	35.27^{a}	64.73^{b}	0.54^{a}
	L.S.D	* 5.38	NS	* 3.92	* 0.116

* (P≤0.05), NS: Not significant.

small letters represents the significances among the means in columns.

Amylose and amylopectin content: (Table 1) shows the amylose and amylopectin content of extracted starch from the studied grains (rice, millet, oats). The amylose values for rice treatments (TR6, TR5, TR4, TR2) were (23.4, 24.6, 24.9, and 26.23) %, respectively. The highest value was in treatment TR2, and the differences among treatments were non-significant. These values were higher than (18.60 %) reported by (Desam *et al*., 2020). The results show a decrease in amylose content with a gradual increase in the concentration of alkaline extraction solution. Wang & Copeland (2012) mentioned that amylose in starch granules is located in the central amorphous core, surrounded by clusters of amylopectin. During the alkaline treatment of starch granules, a limited form of gelatinization occurs. When the alkaline solution comes into contact with starch granules, it can break the hydrogen bonds of amylopectin, leading to a change in the structure of the granules, which gradually disintegrate and seem to fuse at the edges, resulting in a loss of amylose and a decrease in crystallinity. The amylose-to-amylopectin ratio can predict the glycemic index. A high amylose/amylopectin ratio indicates a lower glycemic index (Verma *et al*., 2018). The same table indicates that amylose percentages for

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millet starches for treatments (TM2, TM4, TM5, TM6) were (37.93, 34.76, 33.31, and 32.33) %, respectively. The highest value was for treatment TM2, which was (37.93%). This is higher than the value reported by (Li *et al*., 2019), which was (32.8%). Magallanes *et al* (2017) reported that the amylose content in pearl millet ranged from (30% to 34%). The variation in amylose content may be attributed to differences in varieties, environmental conditions, starch extraction methods, or the methods used for its estimation (Shah *et al*., 2017). It is also observed from the table above that amylose percentages for oat starch in treatments (TO2, TO4, TO5, TO6) were (35.27, 31.22, 30.74, and 30.32) %, respectively. The highest value was recorded in treatment TO2 (35.27%), which is significantly higher than the values recorded by other treatments. This is higher than the values reported by (Mirmoghtadaie *et al*., 2009), which ranged from (27.5 to 29.5) %. Additionally, Punia *et al* (2020) noted that amylose percentage (16.7 - 22) % decreases due to forming a fat-starch complex. As amylose percentages without complex formation were found to be, (19.4 to 33.6) % in oat starch. This is consistent with Khatoniar & Das (2020), who attributed these differences to the interference of unextracted fats in the amylose-fat complex, which may lead to a reduction in amylose content.

Chemical composition of grains and starch

(Table 2) shows the chemical composition of the flours of the studied crops (rice, millet and oats). The percentages of fat, protein, carbohydrate, ash, fiber and moisture in rice flour were (1.09, 3.28, 85.77, 0.64, 1.34 and 7.88) %, respectively. Shakri *et al* (2021) reported that rice grains contained approximately (5.96%) protein, (1.24%) fat, (7.07%) fiber, (0.39%) ash and 80.14% starch. For millet flour, the percentages were (3.31, 7.71, 74.22, 1.57, 1.61 and 11.57) %, respectively, which are different from the values reported by (Das *et al.,* 2019) which were (3.5%) fat, (11%) protein, (56.1%) carbohydrate, (3.6%) ash, and 9% fiber. The chemical composition of oat flour was (6.48, 11.19, 63.2, 1.53, and 8.45) % for fat, protein, carbohydrate, ash, and fiber respectively. The same source reported that oat grains contained (6.4, 17.1, 52.8, 3.2, and 11.3) % of these components, respectively. These differences may be attributed to differences in cultivars, agricultural practices, or environmental conditions. (Table 3) shows the chemical composition of starch extracted from grains using the alkaline method. The moisture content ranged from (8.9 to 10.7) % in the types of extracted starch. Raajeswari *et al* (2023) indicated that the optimum moisture content for long-term storage of extracted starch should be between (8-12%). The protein content of rice starch was (0.3%) to (0.34%), which is lower than the 0.4% which reported by (L. Wang *et al*., 2020), who used a (0.4%) alkaline solution for extraction. For millet starch, the protein content was (0.29% to 0.32%), which was higher than the (0.26%) reported by (Punia *et al*., 2020) when using a (0.3%) alkaline solution for extraction. For oat starch, the protein content ranged from (0.89% to 0.97%), which is in line with (0.13% to 0.95%) reported by (Kaur *et al*., 2022). All results showed no significant difference. The ash content was inversely proportional to the amount of extracted starch, indicating the efficiency of the alkaline extraction process. High efficiency of starch extraction is characterized by low protein, fat, and ash content (Arns *et al*., 2015). Bindar & Efan (2013) observed that the quality of the starch produced is characterized by high starch content and low protein content, as the alkaline solution disperses the protein matrix, leaving the starch free of protein. Increasing the concentration of the alkaline solution leads to a decrease in the protein content, as shown in the results in Table (3). For all treatments. The fat content in the extracted starch was low and not significant for all treatments. For rice starch, it ranged from (0.28% to 0.35%), which is lower than (0.4%) reported by (Bindar & Efan, 2013). In millet starch, the fat content ranged from (0.04 to 0.05) %, lower than (0.27%) reported by (Singh *et al*., 2003). For oat starch, the fat content ranged from (0.11 to 0.16) %, whereas (21) reported a fat content of (0.5%). This indicates the effectiveness of alkaline treatment in removing fat, likely due to partial saponification that

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enhances the removal of residual fatty acids (12). The results in (Table 3) illustrated that the highest fiber content (1.5%) was found in millet starch for the treatment TM6. The lowest fiber content (0.55%) was observed in oat starch for treatment TO5. Wijesinghe & Gunathilake (2020) found fiber contents of (0.12%) and (0.13%) in rice and millet starch, respectively, using an alkaline solution with a concentration of (0.25%). Mahajan et al (2021) noted that millet content of fiber is significantly higher as compared to other grains. (Table 3) shows starch is notably affected by alkaline solution concentration as compared to rice and millet. This is due to what was mentioned by Zwer (2010) that oats contain soluble fiber primarily in the form of β-glucan, which is a highly viscous carbohydrate found in small amounts in the aleurone cell walls; there are also significant concentrations in the endosperm cell walls, where it constitutes approximately 55% of the dietary fiber of oats, with the remaining 45% being insoluble fiber. Beta-glucanase present in the aleurone cell walls may improve the water-binding ability of oat bran, which improves its effectiveness as a dietary fiber. Due to its solubility, the total fiber content of starch decreased as a result of treating oat flour with an alkali solution, while rice and millet do not contain this type of fiber.

Table 2. Chemical Composition of Grain Flours (Rice, Millet, Oats)

Grains	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)	Fiber (%)	Moisture (%)
Rice	1.09	3.28	85.77	0.64	1.34	7.889
Millet	3.31	7.71	74.22	1.57	1.61	11.575
Oat	6.48	11.19	63.2	1.53	8.45	9.15

Table 3. Chemical composition of starch extracted from rice, millet & oats using alkaline method

	Treatments	Moisture Content (%)	Fat Content (%)	Protein Content (%)	Ash Content (%)	Fiber Content (%)	Carbohydrate Content (%)
Rice	TR6	8.9^{a}	0.28^{a}	0.3^{a}	0.22^{a}	1.4^{a}	88.9^{a}
	TR5	10.4^{a}	0.29^{a}	0.33^{a}	0.20^{a}	1.34^{a}	87.44^{a}
	TR4	10.7^{a}	0.31^{a}	0.33^{a}	0.18^{a}	1.3^{a}	87.18^{a}
	TR2	9.9^{a}	0.35^{a}	0.34^{a}	0.20^{a}	1.31^{a}	87.9^{a}
	L.S.D.	NS	NS	NS	NS	NS	NS
Millet	TM6	10.3^{a}	0.04^{a}	0.31^{a}	0.97^{a}	1.5^{a}	86.88^{a}
	TM5	10.1^{a}	0.05^{a}	0.31^{a}	0.92^{a}	1.14^{b}	87.48^{a}
	TM4	9.8^{a}	0.05^{a}	0.29^{a}	0.89^{a}	1.1^{b}	87.87^{a}
	TM2	9.7^{a}	0.04^{a}	0.32^{a}	0.86^{a}	1.05^{b}	88.03^{a}
	L.S.D.	NS	NS	NS	NS	NS	NS
Oats	TO6	10.6^{a}	0.11^{a}	0.89^{a}	0.85^{a}	0.96^{a}	86.59^{a}
	TO5	10.6^{a}	0.14^{a}	0.91^{a}	0.34^{b}	0.59^{b}	87.42^{a}
	TO4	9.5^{a}	0.15^{a}	0.93^{a}	0.25^{b}	0.55^{b}	88.62^{a}
	TO2	10.4^{a}	0.16^{a}	0.97^{a}	0.15^{b}	0.62^{b}	87.7^{a}
	L.S.D.	NS	* 0.058	NS	* 0.39	* 0.316	NS

* (P≤0.05), NS: Not significant.

small letters represents the significances among the means in columns

Functional properties

Swelling capacity: (Table 4) shows the swelling capacity of the extracted rice, millet and oat starch. All treatments showed a significant increase in swelling capacity with increasing temperature (up to 95 °C) as the highest values recorded at this temperature. The results indicated that the extracted rice starch (TR6, TR5 and TR4 treatments) possessed the highest swelling capacity, being (10.34, 10.12 and 9.45) %, respectively. The highest value in this study was lower than the value reported by (Wang et al., 2020) which was 11.7%. Mirmoghtadaie *et al* (2009) reported that increasing the temperature of the medium leads to increased mobility of starch granules which consequently enhance water penetration. Wani *et al* (2012) observed that swelling and melting indices reflect the degree of interaction between starch chains within the

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amorphous and crystalline regions, and this interaction is affected by the amylose/amylopectin ratio and the molecular structure of amylopectin.

Table 4. Swelling capacity of starch extracted from rice, millet and oats by the alkaline method

	treatments	Swelling capacity% 55c°	60 c°	70 c°	80 c°	90 c°	95 c°	L.S.D.
Rice	TR6	$^{D}1.31^{b}$	$^{D}1.89^{a}$	$^{C}6.47^{a}$	$^{B}8.59^{a}$	$^{A}9.91^{a}$	$^{A}10.34^{a}$	1.29 *
	TR5	$^{D}1.32^{b}$	$^{D}1.78^{a}$	$^{C}6.22^{a}$	$^{B}8.05^{a}$	A9.66a	$^{A}10.12^{a}$	1.44 *
	TR4	$^{D}1.25^{b}$	D1.65^{a}	$^{C}6.14^{a}$	$^{B}8.042^{a}$	$^{A}9.21^{a}$	$^{A}9.45^{a}$	1.07 *
	TR2	$^{D}1.84^{a}$	D1.46^{a}	C5.76^{a}	$^{B}7.68^{a}$	$^{A}B8.78^{a}$	$^{A}9.24^{a}$	1.52 *
	L.S.D	NS	NS	0.65 *	0.802 *	0.902 *	0.966 *	L.S.D
Millet	TM6	$^{D}0.76^{a}$	$^{D}0.91^{a}$	$^{C}2.87^{a}$	$^{B}4.84^{a}$	$^{A}7.32^{a}$	$^{A}8.11^{a}$	1.08 *
	TM5	$^{C}0.95^{a}$	$^{C}1.28^{a}$	$^{C}2.13^{a}$	$^{B}4.72^{a}$	^{A}B5.13^{b}	$^{A}6.25^{b}$	1.17 *
	TM4	$^{D}0.71^{a}$	$^{D}0.87^{a}$	$^{C}2.85^{a}$	$^{B}4.47^{a}$	$^{A}5.64^{b}$	$^{A}6.49^{b}$	0.976 *
	TM2	$^{D}0.69^{a}$	$^{D}0.80^{a}$	$^{C}2.64^{a}$	$^{B}4.5^{a}$	$^{AB}5.54^{b}$	$^{A}6.42^{b}$	1.165 *
	L.S.D	NS	0.422 *	0.476 *	NS	0.952 *	0.885 *	L.S.D
Oat	TO6	$^{C}4.02^{a}$	$^{C}4.55^{a}$	$^{C}5.05^{a}$	$^{BC}6.14^{a}$	$^{A}8.38^{a}$	$^{A}.897^{a}$	1.19 *
	TO5	$^{D}3.96^{a}$	$^{CD}4.49^{a}$	$^{C}5.23^{a}$	$^{B}6.77^{a}$	$^{AB}7.59^{a}$	$^{A}.823^{a}$	0.977 *
	TO4	$^{D}3.89^{a}$	$^{D}4.33^{a}$	$^{CD}5.12^{a}$	$^{BC}5.91^{a}$	$^{B}6.35^{b}$	^{A}7.54a^{b}	1.06 *
	TO2	$^{C}3.86^{a}$	$^{C}4.36^{a}$	$^{C}4.53^{b}$	$^{B}5.77^{a}$	$^{AB}6.23^{b}$	$^{A}6.96^{b}$	1.18 *
	L.S.D.	NS	NS	NS	NS	0.91 *	0.94 *	---

.Not significant :NS ‹(P≤0.05) *

Capital letters represents the significances among the means in rows.
small letters represents the significances among the means in columns

For millet starch, all treatments showed a significant increase in swelling capacity with rising temperatures (up to 95°C). The highest swelling capacity (8.11%) was observed for TM6 treatment at 95°C, which is lower than the value (16%) reported by (Wen *et al.*, 2014). Regarding the oat starch in the same table the highest value for swelling capacities for TM6 treatment was (8.97%) at 95°C, which is lower than the value (19.63%) reported by (Muhammad Usman *et al.*, 2014). Olu-Owolabi *et al* (2011) suggested that increasing the temperature weakens binding forces within the starch granules, facilitating the entry of water into crystalline regions of starch, thereby causing an increase in the swelling of the starch granules. Hoover *et al* (2003) found an inverse relationship between swelling capacity and the amount of amylose-lipid complexes and long amylopectin chains, which inhibit swelling capacity. The binding of long amylopectin chains can lead to the formation of numerous crystals, which may increase the stability of granules and thus reduce their swelling capacity.

Solubility: (Table 5) shows the solubility properties of starch extracted from rice, millet and oats. The values varied and increased in importance with increasing temperature. The highest solubility value was observed for millet starch (3.28%) at 60°C, followed by rice starch (3.15%) at 70°C, recorded for TR4 treatment. This is higher than that reported by (N. Singh *et al.*, 2003), where the values ranged from 0.287-0.360%. For millet, Singh & Adedeji (2014) reported that the solubility value of starch was 2.62% at 70°C, which is close to that of TM5 (in this study) treatment at the same temperature .

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Table 5. Solubility values of starch extracted from rice, millet and oats using the alkaline method

	Treatments	Solubility % 55c°	60 c°	70 c°	80 c°	90 c°	95 c°	L.S.D.
Rice	TR6	^{B}0.97 ^{b}	^{A}1.69 ^{b}	^{A}2.16 ^{b}	^{AB}1.46 ^{a}	^{B}0.80 ^{b}	^{B}.053 ^{b}	0.721 *
	TR5	^{A}2.35 ^{ab}	^{A}2.76 ^{ab}	^{A}3.10 ^{ab}	^{B}1.21 ^{ab}	^{B}1.01 ^{b}	^{B}0.52 ^{b}	0.825 *
	TR4	^{A}2.61 ^{a}	^{A}2.87 ^{a}	^{A}3.15 ^{a}	^{B}0.76 ^{b}	^{B}0.60 ^{b}	^{B}0.49 ^{b}	0.755 *
	TR2	^{A}0.58 ^{b}	^{A} 0.65 ^{c}	^{A}0.85 ^{c}	^{A}1.09^{ab}	^{A}1.73 ^{a}	^{A}1.12 ^{a}	0.539 *
	L.S.D	0.537 *	0.851 *	0.859 *	0.486 *	0.503 *	0.489 *	L.S.D
Millet	TM6	^{C}0.72 ^{b}	^{C}0.84 ^{b}	^{C}1.17 ^{b}	^{A}2.79 ^{a}	^{BC}1.72 ^{ab}	^{B}1.94 ^{a}	0.571 *
	TM5	^{D}1.86 ^{a}	^{A}3.28 ^{a}	^{C}2.31 ^{a}	^{B}2.73^{ab}	^{E}1.23^{ab}	^{E}1.03 ^{b}	0.463 *
	TM4	^{B}0.60 ^{b}	^{B}0.75 ^{b}	^{B}0.84 ^{b}	^{B}0.29 ^{b}	^{A}1.75 ^{a}	^{B}1.23 ^{b}	0.498 *
	TM2	^{A}.055 ^{b}	A0.67 ^{b}	^{A}0.97 ^{b}	^{A}0.40 ^{b}	^{A}0.62 ^{b}	^{A}0.55 ^{b}	0.422 NS
	L.S.D	0.511 *	0.704 *	0.622 *	0.894 *	0.647 *	0.702 *	L.S.D
Oat	TO6	^{C}0.98 ^{a}	^{BC}1.14 ^{a}	^{C}1.00 ^{ab}	^{A}2.37 ^{a}	^{B}1.66 ^{a}	^{C}1.01 ^{a}	0.655 *
	TO5	^{A}0.59 ^{a}	^{A}0.73^{ab}	^{A}0.61 ^{b}	^{A}1.07 ^{b}	^{A}0.97 ^{ab}	^{A}0.44 ^{ab}	0.492 *
	TO4	^{A}0.91 ^{a}	^{A}0.82 ^{ab}	^{A}0.88 ^{b}	^{A}1.36 ^{b}	^{A}0.80 ^{ab}	^{A}0.61 ^{ab}	0.596 *
	TO2	^{B}0.64 ^{a}	^{B}0.55 ^{b}	^{A}1.38 ^{a}	^{AB}0.98 ^{b}	^{B}0.56 ^{ab}	^{B}0.46 ^{ab}	0.544 *
	L.S.D.	NS	0.527 *	0.495 *	0.792 *	0.834 *	0.527 *	---

Not significant :NS ‹(P≤0.05) *

Capital letters represents the significances among the means in rows.
small letters represents the significances among the means in columns

The highest solubility value (2.37%) for oat starch was recorded for TM6 treatment at 80°C, this is lower than that reported (2.80%) by (Berski et al., 2014) at the same temperature. Bangar *et al* (2021) mentioned that solubility is influenced by the components and size of starch granules, crystalline arrangement, gelatinization extent, and starch granule formation. Wani *et al* (2012) noted that heating starch with excess water disrupts the crystalline structure of starch due to breaking of hydrogen bonds, leading to new bonds forming between the OH groups of water and starch, which increases swelling capacity and solubility. Additionally, the distribution of amylose and amylopectin in starch granules also affects solubility. Amylose plays a role in maintaining the granule structure and is concentrated in the center of the granule. Therefore, higher amylose content increases granule density, making it harder for starch to leach out of the granules and thus reducing solubility values.

Water Holding Capacity: (Table 6) shows the water binding capacity and pH of starch extracted from rice, millet and oats. The water binding capacity of rice starch ranged from (93.26 to 124.06) %, the highest value recorded by TR6 treatment, which is lower than the range reported by (Qadir *et al.*, 2021), which was between (183.33%) and (191.41%).In millet starch, the water binding capacity ranged from (74.18 to 154.62) %. The highest value for treatment TM5 exceeded the value (138.43 ± 1.93%) reported by (Singh & Adedeji, 2017). The capacity of starch to bind water reflects the bonding strength within starch granules. Additionally, an increase in amylose content in starch helps maintain granule structure, leading to a decrease in water binding capacity (Nakorn *et al.*, 2009). For oat starch, the water binding capacity ranged from (73.29 to 121.72) %, with a significant difference among treatments. The highest value for treatment TO5 (121.72%) was greater than (100.0% ± 1) that reported by (Kumar *et al.*, 2018).

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Table 6. Water binding capacity, oil holding capacity, and pH of starch extracted from rice, millet, and oats using the alkaline method.

	Treatments	Water holding capacity (%)	Oil holding capacity (%)	pH
Rice	TR6	124.06^{a}	170.51^{a}	6.6^{ab}
	TR5	115.36^{a}	154.93^{a}	6.9^{ab}
	TR4	93.26^{b}	132.99^{b}	6.8^{ab}
	TR2	107.45^{ab}	161.58^{a}	6.9^{a}
	L.S.D	17.94 *	18.04 *	NS
Millet	TM6	108.59^{b}	123.59^{ab}	6.9^{a}
	TM5	154.62^{a}	130.34^{a}	6.81^{a}
	TM4	74.18^{c}	132.16^{a}	6.6^{a}
	TM2	76.95^{c}	111.15^{a}	7.0^{a}
	L.S.D	15.04 *	14.97 *	NS
oat	TO6	94.85^{bc}	149.85^{a}	6.8^{a}
	TO5	121.72^{a}	143.60^{a}	7.0^{a}
	TO4	97.36^{b}	144.05^{a}	6.8^{a}
	TO2	73.29^{c}	158.18^{a}	6.9^{a}
	L.S.D.	15.63 *	14.19 *	NS

***small letters represents the significances among the means in columns**

Differences in water binding capacity results across studies may be attributed to variations in molecular structure, crystalline versus amorphous regions within the starch granules, and granule size distribution (Shah *et al.*, 2017). Nakorn et al (2009) indicated that the capacity of starch to bind water expresses the extent of the cohesive forces in the starch granules. Also, the increase in the amylose content in starch plays a role in maintaining the granular structure and thus a decrease in the water binding capacity. As for Al-Mahyawi (2023), indicated that flour with high water absorption contains hydrophilic components such as polysaccharides and protein that has a hydrophilic nature and another that hydrophobic and that the increase in the water-binding capacity has always been associated with increased amylose filtration, solubility, and loss of the crystalline structure of the starch. The difference in the water-holding capacity of different types of flour may be due to the difference in protein concentration and its ability to interact with water, and the rate of protein absorption of water is affected by several factors, including the molecule containing additional side groups such as amine, carboxyl, and hydroxyl groups, and other factors such as pH, ionic strength, and temperature.

Oil holding capacity: (Table 6) shows the percentage of oil holding capacity for each starch sample (rice, millet, and oats) extracted using an alkaline method, the results indicated that there were significant differences among the treatments. The highest oil holding capacity for rice starch was recorded for treatment TR6, which reached (170.51%), while the lowest was (132.99%) for treatment TR4. All values were lower than those reported by Bhat & Riar (2016), who found rice starch oil holding capacities ranging from (245.33% to 294.33%).For millet starch, the oil holding capacity ranged from (111.15% to 132.16%), indicating that each gram of millet starch could hold (1.11 to 1.32) grams of oil. In comparison, the oil holding capacity for millet flour was (1.16) grams of oil per gram of flour as reported by (Khatoniar & Das, 2020).The oil holding capacity is primarily attributed to the physical retention of oil within the starch matrix due to the lack of non-polar sites in the starch, such as proteins. Proteins have both hydrophilic and hydrophobic bonds which influence water and oil binding properties. Additionally, millet's high fiber content reduces oil absorption, which may weaken its ability to hold oil in food formulations (Mahajan *et al.*, 2021).In the same table, the oil holding capacities for oat starch samples ranged from (143.60 to 158.18) %, with no significant difference among the treatments. These results were higher than those reported by (Falsafi *et al.*, 2019), who found oil holding capacities between (41.43 and 57.57) %. This researcher attributed the starch granules' ability to retain oil to two main reasons: one being the filling

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of spaces between starch granules with oil, and the other being the trapping of oil particles in the fibers within the channels and pores on the surface of the starch granules, This results in an increase in the capillary attraction of oil particles.

CONCLUSION

The study showed that extraction with an alkaline solution of (0.4)% for rice flour starch and (0.2)% for millet and oat flour starch recorded the highest final yield with the morphological integrity of the starch granule. The increase in the concentration of the alkaline solution leads to alkaline gelatinization and also a decrease in the amylose content of the extracted starch and its effusion outside the starch granule. There is also an inverse relationship between the percentage of amylose and the percentage of swelling capacity, solubility and water carrying capacity of the starch granule.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

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تأثير الاستخلاص القلوي على الخصائص الوظيفية والفيزيوكيميائية للنشا المستخلص من الأرز والدخن والشوفان

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المستخلص

هدفت هذه الدراسة الى تقصي تأثير الاستخلاص القلوي باستعمال تراكيز مختلفة من محلول هيدروكسيد الصوديوم (NaOH) (0.6 ، 0.5 ، 0.4 ، 0.2) % على الخصائص الفيزيوكيميائية والوظيفية والحصيلة النهائية للنشا المستخلص من دقيق الارز والدخن والشوفان. أظهرت النتائج ان حصيلة النشا المعزول من دقيق الارز كانت تتراوح (50 – 68.18 % وإن أعلى عائد من النشأ تحقق بأستخدام محلول القلوي (NaOH) بتركيز 0.4%، أما في محصولي الدخن و الشوفان فقد تراوح عائد النشا (47.46 – 54)% و (48.3 – 62.66)% على التوالي، و أعلى حصيلة كانت عند تركيز (0.2) % لكلا المحصولين. كانت النسبة المئوية للأميلوز مرتفعة في نشأ الحبوب عند أستعمال التراكيز المنخفضة من المحلول القلوي. ارتبطت نسبة الاميلوز عكسيا مع تركيز المحلول القلوي ومع الخواص الوظيفية (سعة الانتفاخ والذوبانية) والتي تزداد عند انخفاض نسبة الاميلوز في النشا، وسجلت اعلى قيم سعة الانتفاخ عند استعمال التراكيز العالية من المحلول القلوي حيث كانت اعلى قيمة سعة الانتفاخ لنشا الارز و نشأ الدخن و نشأ الشوفان (10.34)% و(8.11)% و(8.97)% على التوالي.

كلمات مفتاحية: الاميلوز، الاميلوبكتين، خصائص فيزيوكيميائية.

***جزء من رسالة الماجستير للباحث الاول.**