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Effect of annealing on the chemical and physico- functional properties of fermented provitamin A cassava starch

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ABSTRACT: This study reports the effect of annealing on chemical and physico-functional properties of fermented pro-vitamin A cassava starch of cassava roots TME-IBA 070539 variety. The provitamin cassava starch flour was extracted to produce native **s**tarch flours (NCS). Fermentation was carried out on the tubers at 24 and 48 hours to produce provitamin A cassava fermented starches FCS24 and FCS48 respectively. The starch flour samples NCS, FCS24 and FCS48 were then annealed at 50°C to produce annealed native starches (ANCS), annealed 24 hours fermented cassava starch (AFCS24) and annealed 48 hours fermented cassava starch (AFCS48). The chemical and physico-functional properties were evaluated using standard methods. The result revealed negligible amount of crude protein and ash content. However, the carbohydrate content in all the samples ranged between 93.39 – 95.01% signifying high purity starches. These processes imparted positively the β- carotene content which ranged between 2.50 – 2.80 µg/100g in the cassava starches. Although, no significant ($p>0.05$) difference was observed in the β-carotene content when compared with the corresponding annealed starches except for a slight reduction at AFCS48. The amylose content decreased significantly ($p>0.05$) by 21.72 and 38% in the combined process of annealing and fermentation as observed for AFCS24 and AFCS48 respectively. Also, amylose leaching was more pronounced in the NCS than the fermented and annealed starches. No significantly ($p<0.05$) difference was observed in the corresponding samples; ANCS, AFCS24 and AFCS48 for total titratable acidity while pH decreased significantly in the annealed starches. The swelling and solubility increased with increase in temperature, generally annealing lowered the swelling capacity and increased the solubility of the starch samples and this could be harnessed in food preparations and in the industries.

Keywords: Cassava starch, annealing, fermentation, chemical properties, functional properties.

INTRODUCTION

Starch is a homo-polysaccharide synthesized in the chloroplast of green leaves. It is the most important and by far the most accessible carbohydrate in the human diet (Nawaz *et al.*, 2020). Starch in its pure form is referred to as “Native Starch” obtained from a variety of carbohydrate sources such as wheat, cassava, potatoes, corn, rice etc (Horstmann *et al.,* 2017). In the food segment, starch is widely used as moisture retainer, stabilizing, texturizer and gelling agent, binders and its suitable for use in edible films production. Cassava (*Manihot esculenta Crantz*), is a staple food crop for over 700 million people of the world living in sub-tropical and the tropical areas (Boakye *et al*., 2020) with a total world production of approximately 302.66 million metric ton volume as of 2020 out of which Nigeria produces about 60 million metric tons as the highest producer of cassava in the world (FAO, 2022). In

Nigeria, cassava foodstuffs are essential part of household food basket (Ikuemonisan *et al.,* 2020). It is also one of the high yielding and prominent source of starch, though this depends on the period of harvest and/or cultivar (Boakye *et al*., 2020).

The white flesh colour cassava is historically known and used widely in manufacturing of high quality white starch flour. The bio-fortified cassava also referred to as yellow-fleshed cassava is a significant initiatives implemented in countries with high cassava consumption in their populations to improve the vitamin A status of a targeted population hence controlling Vitamin A deficiency (VAD) occurrence which remains a big challenge for nutritionists and health workers (Das *et al*., 2013; Udoh *et al*., 2022). These biofortified provitamin A cassava is genetically improved for increased provitamin A content. The carotene content is higher in these varieties and noticeable deeper colour intensity and is commonly referred to as 'yellow cassava' (Ayetigbo *et al*., 2018).

Fermentation is an age long and complex metabolic and efficient food biotechnology process by which enzymes/microorganisms convert carbohydrates into various desirable biochemicals (Sharma *et al*., 2020). Fermentation is one of the most convenient and widely used methods in food processing especially in cassava processing even in the rural areas. It has been widely used to improve food properties either with or without addition of culture and the quality characteristics has been dependence on factors such as the microorganisms involved and the fermentation period (Alexander Essers, 1994).

Cassava starch in its native state has drawbacks when used in food processing. Therefore, there is need for modification to improve the functionalities and easy utilization of starches for various industrial applications (Oyeyinka and Oyeyinka, 2018). Different modification methods such as chemical, physical and a combination of these methods are used in starch modification (Nawaz *et al.,* 2020). Modification process brings starch temperature to a value that would result in the homogeneity of the crystalline structure of starch and also the motility of starch molecules but not to the extent of starch transformation into a paste thereby protecting the structure of granules (Zia-ud-Din *et al*., 2015; Rocha *et al*., 2012).

Annealing is one of the physical methods used in the modification of starch so as to overcome the inherent limitations of native starch's poor resistance in extreme processing conditions of heat, shear, syneresis and retrogradation when reheated or in acidic environment as commonly encountered in the industry (Pérez *et al*., 2011). Basically, annealing treatment carried out in excess (60 w/w) or intermediate (45-50 w/w) moisture contents modifies the physicochemical properties of starch without affecting the starch granule structure because the annealing temperature is below gelatinization temperature (Alvani *et al*., 2012; Tester and Debon, 2000).

A new source of starch is continuously being explored from non-conventional sources so as to broaden its utility in food product development and applications in industries. In Nigeria, the demand for cassava starch exceeds 350,000 tons per year, with only around 20% of the estimated production potential being met. Previous studies (Gomes *et al*., 2005; Dolas *et al*., 2020; Sudhakara *et al.,* 2023) have focused on annealed starches from white cassava but studies on the more recently released yellow cassava cultivar is sparse. More importantly, effect of modification such as annealing of yellow cassava starch is rare. Pro-Vitamin A cassava commonly referred to as yellow cassava's utilization has been restricted to consumption and production of local foods. With the growing interest in the use of cassava starch in food product development, this research will fill the gap with respect to information regarding annealing and fermentation and the effect on the chemical and functional properties of yellow cassava starch. This annealing process which will modify cassava starch below its gelatinization temperature (60-80°C) would enhance the development of value-addition of yellow cassava and its utilization in food products which may fill in the demand and at the same time impart novel characteristics in food applications. It is however rationale to investigate the quality of the starch from different processing conditions which will enhance a wider range of application in the industries. This study could provide a convincing entry point for pro-vitamin cassava starch commercialization, utilization and overall increase in production of these crops. Provision of experimental data results of the starches can be a better alternative in food where starch is needed and could be suitable for the intended applications.

MATERIALS AND METHODS

Materials

Pro-vitamin A cassava variety of TME-IBA 070539 of five kilograms (5 kg) was procured from the International Fund for Agricultural Development (IFAD), Bida, Niger State, Nigeria.

Production of fermented and unfermented yellow cassava starch flours

The cassava starch was extracted according to the wet milling method described by Paixão E Silva *et al*. (2021) with slight modification. The provitamin A cassava tubers were cut to about 2 cm thick slices after being peeled using a sharp stainless knife. This was then separated into three (3) batches. One batch was milled to a pulp and floated and agitated in ten times its water volume and filtered

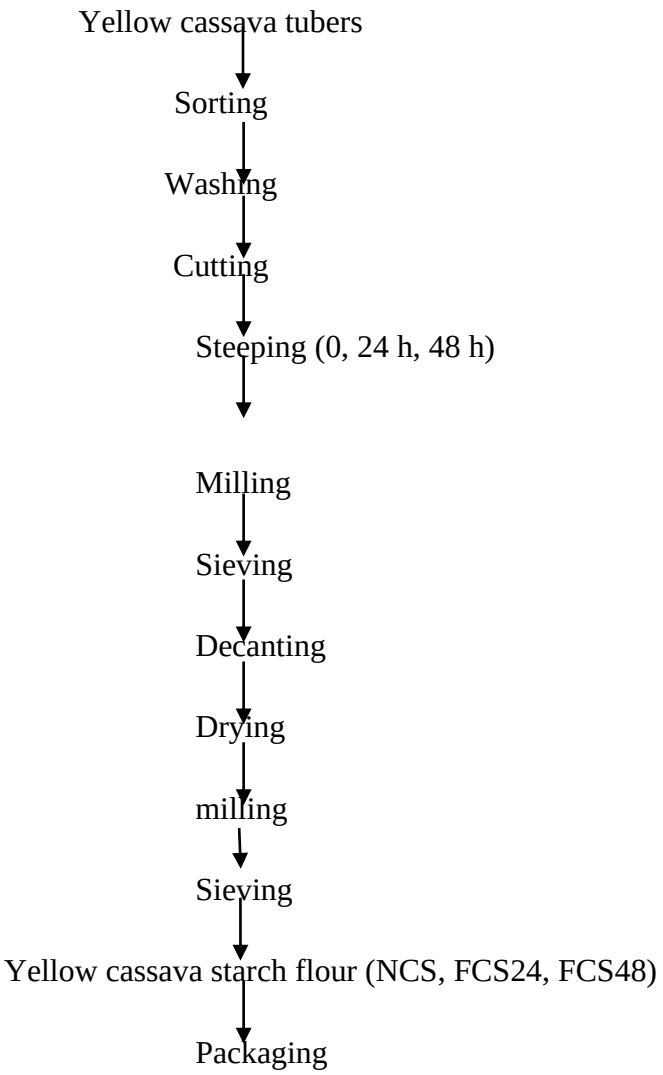

Figure 1. Production of fermented and unfermented pro-vitamin A cassava starch. **Key:** NCS – Native cassava starch; FCS24 – 24 hours fermented cassava starch; FCS48 – 48 hours fermented cassava starch (Source: Paixão E Silva *et al*., 2021 modified).

using a double-layered cheese cloth. The filtration process was repeated and the filtrate was allowed to stand for 2 hours to facilitate starch sedimentation and then the supernatant was decanted. This starch paste was dried in air forced oven at 50°C for 48 hours to obtain the native cassava starch flour (NCS). Natural fermentation was carried out on the remaining two batches by steeping the cassava tubers in water (1:2 w/v) and the tubers were left to naturally ferment for 24 and 48 hours respectively. Thereafter, water was drained and the fermented cassava milled to a pulp, sieved and the cassava starch allowed to settle. The fermented cassava paste was recovered and dried using an air forced oven at 50°C for 48 hours to obtain fermented yellow cassava flour at 24 (FCS24) and 48 hours (FCS48) respectively (Figure 1).

Annealing of pro-vitamin A cassava starch

Annealing of the various cassava yellow starches was carried out using the routine described by Ikegwu *et al*. (2011). About 100 g each of NCS, FCS24 and FCS48 flour samples were mixed with 200 ml of distilled water and the mixture was incubated at 50°C for 24 hours and the suspension was filtered using a Whattman No 1 filter paper. The filtrate was oven dried at 50°C and milled using a blender (Phillip Mode lHR2102), sieved to obtain annealed unfermented cassava starch (ANCS), 24 hours fermented annealed cassava starch (AFCS24), and 48 hours fermented annealed cassava starch (AFCS48).

Determination of chemical properties of the starch samples

Determination of proximate composition

Determination of proximate composition: The method described by AOAC (2012) was employed for the proximate analysis. The moisture content was determined by the oven method, where the starch samples were placed in the oven at 105°C until constant weight was recorded. The Kjeldahl method was used in determining the crude protein content where the Nitrogen content was determined and coefficient 6.25 was used in the conversion to crude protein. The total lipid was determined by continuous solvent extraction in soxhlet apparatus for 8 hours, ash was determined using muffle furnace at 550°C for 16 hours and the incombustible content was weighed and reported. The crude fiber was determined by sequential chemical digestion. Carbohydrate content was evaluated by difference (Onwuka, 2005) as stated below;

% Carbohydrate = 100 - (Moisture content + crude protein + crude fat + ash + crude fibre)

Determination of amylose content

Amylose content was evaluated using the protocol described by Nuwamanya *et al*. (2015). Essentially, impurities were removed by dispersing 100 mg of starch into 1 ml of hot ethanol and allowed to stand for 2 minutes. The ethanol was decanted and the starch was gelatinized by heating for 30 minutes in 5 ml of 0.1M sodium hydroxide at 80°C in a water bath. Aliquot of 0.1 ml was obtained from the gelatinized solution and an equal volume of 0.1 M acetic acid was added. The solution was then diluted with 4.6 ml of distilled water followed by staining with 0.2 ml of 10% iodine/KI solution. The concentration of amylose was then determined by reading the absorbance of the stained

solution using a spectrophotometer at 620 nm. The amylose content (%) was calculated using an amylose standard from Sigma Aldrich.

Determination of amylose leaching

Essentially, 10 ml of distilled water was added to starch sample of 100 mg, and heated in water bath (Thermostatic Water Bath Gallenkemp, England) for 30 min at two different temperatures of 70 and 80°C. The samples were agitated all through the heating period to sustain the starch suspension. This was followed by centrifuging (Centrifuge Model 80-2, Lenfield Medical, England) at 1500 rpm for 10 min. Subsequently, 5 ml of the supernatant was introduced to a standard flask of 100 ml and 1 ml of acetic acid with 2 ml iodine was introduced. The volume was then made up to 100 ml mark with distilled water, shaken and the absorbance was determined at 620 nm against blank using UV- spectrophotometer.

Determination of β-carotene content

The β-carotene content was determined using the AOAC (2012) protocol. Into a conical flask containing 50 ml of 95% ethanol, 5 g of each starch sample was weighed and placed in a water bath at a temperature of 80°C for 20 minutes with periodic agitation. The supernatant was decanted, allowed to cool and the volume was recorded as initial volume. Thereafter, 15 ml of distilled water was added to bring the ethanol concentration of the mixture to 85% and it was further cooled in a container of ice water for about 5 minutes. The mixture was transferred in to a separating funnel and 25 ml of petroleum ether was added and the cooled ethanol was poured over it. The funnel was swirled gently to obtain a homogenous mixture and then allowed to stand until two separate layers were achieved and the top layer was collected into a 250 ml conical flask. The bottom layer was collected into a beaker and transferred back into the separating funnel and re-extracted again with 10 ml petroleum ether for 5-6 times until the extract became faint yellow. The entire petroleum ether was collected in to 250 ml conical flask and transferred in to separating funnel for re-extraction with 50 ml of 80% ethanol. The final extract was measured and stored in sample bottles for further analysis. The absorbance of the extracts was measured using a spectrophotometer (model 22UV/VIS) at a wavelength of 436 nm and cuvette containing pet-ether (blank) was used to calibrate the spectrophotometer to zero point. Samples of each extract were placed in cuvettes and readings were taken when the figure in the display window became steady. The β-carotene concentration was calculated using the equation.

$A \propto L$ (if concentration(C) is constant).

$A = ECL$; $C = A/EL$

Where: C = concentration of carotene, A = absorbance, E = extinction coefficient, L = thickness of cuvettes (path length) = 1 cm

E of β-carotene $= 1.25 \times 10^4$ µg/l

Determination of pH and total titratable acidity of the starch samples

The pH was determined by using a handheld digital pH meter (Jenway 3505, Bibby scientific) while the titratable acid (TTA) content was evaluated using the method described by Oyewole and Afolami (2001).

Color determination

The colour of the starch samples was measured with a CHNSpec CS-10 (HangZhouCHNSpec Co. Ltd). The colorimeter operates on the CIE (Commission International de l' Edairage) L∗, a∗, b∗ colour scheme. The colorimeter was calibrated against a standard white reference tile standardized. About 5 g each of the various starch samples were placed on a clean paper and the colorimeters sensor was allowed to touch the samples and the reading for L∗ was measured directly. The instrument displays three – dimensional color differences in uniform color space (lab) coordinates. The three dimensions of light to dark direction called L∗, a red to green direction called a∗, and a blue to yellow direction called b∗ were defined and triplicate readings were taken.

Determination of functional properties

Determination of the swelling capacity, solubility and least gelation capacity

The swelling capacity and solubility of the starches at temperatures of 50, 60, 70, 80 and 90°C was determined according to the method outlined by Hirsch and Kokini (2002) while the method described by Ojinnaka *et al.* (2016) was adopted in the determination of least gelation capacity.

Statistical analysis

Results of all analysis were expressed as the means of triplicate value. Data were subjected using the One-way Analysis of Variance (ANOVA) test and the Duncan Multiple Range Test (DMRT) was used to determine where significant difference existed among the samples. All statistical analyses was carried out by using IBM SPSS Statistical Package (version 20.0).

Table 1. Effect of annealing on the chemical properties of fermented and unfermented pro-vitamin A Cassava Starch.

Parameters (%)	NCS	FCS24	FCS48	ANCS	AFCS24	AFCS48
Moisture content	5.65^{b}±0.04	4.59^{e}±0.01	4.39^{f}±0.01	5.77^{c}±0.01	5.80^{a}±0.00	5.49^{d}±0.01
Crude protein	0.10^{c}±0.00	0.24^{a}±0.01	0.20^{b}±0.01	0.10^{c}±0.01	0.20^{b}±0.01	0.20^{b}±0.01
Ash	0.10^{c} ±0.01	0.39^{b}±0.02	0.40^{b}±0.01	0.10^{c}±0.02	0.40^{b}±0.02	0.48^{a}±0.02
Crude fiber	0.50^{a}±0.00	0.01^{b}±0.00	ND	ND	ND	ND
Carbohydrate	93.39^{f}±0.02	94.77^{b}±0.01	95.01^{a}±0.01	94.03^{c}±0.01	93.60^{e}±0.01	93.83^{d}±0.01
Amylose	29.37^{a}±0.02	27.10^{b}±0.00	22.66^{d}±0.24	26.31^{c}±0.10	22.99^{d}±0.20	18.21^{e}±0.02
B-carotene (µg/100 g)	2.50^{d}±0.01	2.52^{c}±0.00	2.80^{a}±0.01	2.50^{d}±0.00	2.52^{c}±0.00	2.78^{b}±0.01

Values are expressed as means of triplicate determinations. Mean±Standard deviation (SD) values followed by the same letters within the row are not significant differences (p>0.05). **Key:** NCS=Native cassava starch FCS24= 24 h fermented cassava starch, FCS48= 48 h fermented cassava starch flour, ANCS= Annealed native cassava starch, AFCS24= Annealed 24 h fermented cassava starch AFCS48= Annealed 48 h fermented cassava starch flour.

RESULTS AND DISCUSSION

Chemical properties of the starch samples

In this study, the chemical properties of the various provitamin A cassava starches were evaluated as proximate composition, amylose, β-carotene content, pH and total titratable acidity. Table 1 summarizes the chemical properties of the various starch samples. The moisture content varied between 4.39 to 5.80% for FCS48 and NCS respectively. Annealing of starch after fermentation increased the moisture content within the range of 22.78 -26.55% while 2.12% increase was observed in the unfermented annealed sample compared with the NCS. The moisture content reduced with fermentation time while annealing increased the moisture content. The decrease in moisture content with increase in fermentation time might be due to the increase in dry matter content during fermentation as a result of microbial proliferation (Desta *et al*., 2021) while increased in moisture content in the annealed samples can be attributed to the addition of water to the substrate in the annealing process. Reduction of moisture content which was directly influenced by the fermentation time and this was also reported by Detsa *et al.* (2021) in banana starch. Nonetheless, the moisture contents values are still within the range of <10% recommended for safe storage and extension of shelf life of flour products in ambient storage (Tortoe *et al*., 2019). The crude protein and ash content ranged between 0.10% (NCS; ANCS) - 0.20% (FCS24) and 0.10% (FCS24) - 0.48% (AFCS48) respectively. Crude fiber was observed in negligible amount. Fermentation rather than annealing had more effect on the crude protein and ash content, as higher crude protein and ash content were observed in the fermented samples than the unfermented ones. This could be attributed to microbial degradation of complex protein thus releasing peptides and amino acids and reduction of antinutrients that binds with proteins and minerals (Nkhata *et al*., 2018: Pranoto *et al*., 2013). The carbohydrate content differs among the samples and ranged between 93.39 - 95.01% for NCS and FCS48 respectively.

High starch content and purity which were expressed in the carbohydrate values, this also indicates the quality of the starch product and also the solvents' effectiveness in removing other materials such as cell wall polysaccharides, inorganic salts and proteins. This is imperative because the presence of these components can affect the functional properties of the starch (Tapia *et al*., 2012; Ojo *et al*., 2022). Amylose content ranged from 18.21% for AFCS48 to 29.37% for ANCS. Significant reduction in amylose content was observed in the combined process of annealing and fermentation evidenced in AFCS24 and AFCS48 with 21.72 and 38% decrease respectively. The range of amylose content that was observed is comparable with the value of 19-25% reported in white fleshed cassava by Nuwamanya *et al*. (2010) but higher than 16.4 - 22.1% reported by Atwijukire *et al*. (2019). These variations could be as a result of the type of microorganisms inherent which alters the composition of raw materials in terms of the mineral elements and vitamins composition during the fermentation processes (Kiczorowski *et al.*, 2022).

High amylose values above 25% such as in NCS, ANCS, FCS24 tends to be more resistant to digestion and thus could offer some health benefits to humans whereas the lower amylose content are more useful in the industries (Atwijukire *et al*., 2019).

Carotenoids are generally associated with colour of crops, and among all the carotenoids, only β-carotene compounds have full vitamin A activity due to its double ended β ionone ring (Udoh *et al*., 2022).There was a slight increase in β-carotene concentration in all the fermented products irrespective of the fermentation period and the β-carotene content ranged from 2.50 to 2.80 µg/100 for the starch samples. It was observed that fermentation rather than annealing increased the β-carotene contents and a slight decrease was observed at the combined fermenta-

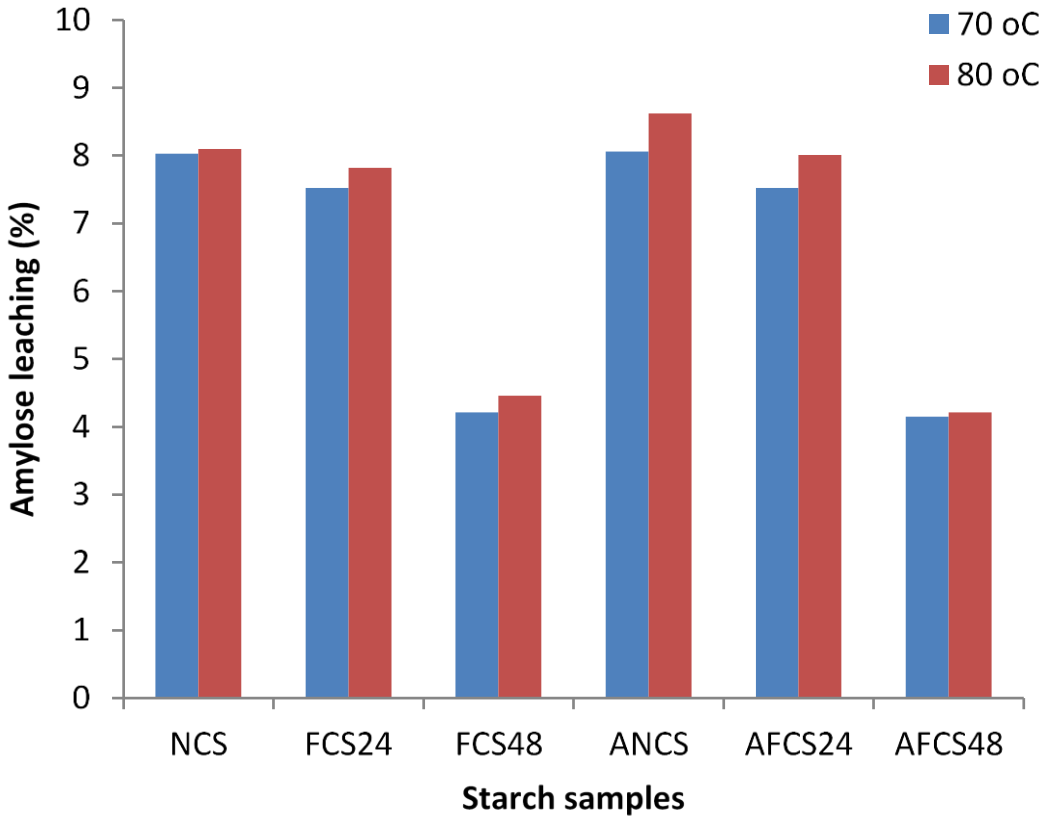

Figure 2. Amylose leaching (%) of the various pro-vitamin cassava starch samples.

tion (48 hours) and annealing (AFCS48). Slight reduction of β-carotene content at AFCS48 could be due to solubility of β–carotene (Demiray and Tulek, 2015).

Effect of temperature on the amylose leaching of the starch samples

The result of the amylose leaching is as illustrated in Figure 2. From the results, increase in temperature (80°C) resulted in increase in amylose leaching in the percentage of 0.87, 2.62, 7.73, 6.95, 6.50 and 1.45%, for NCS, FCS48, FCS24, ANCS AFCS24, and AFCS48 respectively. During amylose leaching several hydrogen bonds between the macromolecules are broken (Nikolenko *et al*., 2023) and a number of factor is reported to influence the process of leaching of amylose from starch granules; this includes presence of polysaccharides, proteins, sugars and salts, the total amylose content as the amount of leaching increases with high amylose content, degree of lipid that complex with the amylose chains and the level in which the amylose chains are associated with each other (Chen *et al*., 2022). The least amylose leaching was observed in sample AFCS48 at both temperatures (70 and 80°C). This could be as a result of low amylose content of the sample.

The pH, Total titratable acidity (TTA) and color characteristics of the starch samples

The pH, TTA and the Hunter colour values (L*, a*, b*) of the starch flour samples as affected by the different treatments are presented in Table 2. The pH, decreased from 5.80 (NCS) to 4.20 (FCS48, AFCS48). There was no significant ($p>0.05$) difference in the pH of FCS48 andAFCS48. The pH of the medium increased distinctly with increased in fermentation time. This could be as a result of the production of organic acids during fermentation. It was observed that annealing did not significantly affect the TTA which ranged between 0.14-0.39 for NCS and AFCS48 respectively. Fermentation rather than annealing significantly influenced the TTA of the starch samples.

An increase of the L*, a* and a decrease of the b* coordinate values, compared with the NCS sample was observed. There was no significant ($p>0.05$) difference between the corresponding annealed sample at the a* values. Among these samples, AFCS was significantly lighter with a high L* of 83.55%, while the highest values of highest greenness (a*) of -1.29 and yellowness (b*) value of 6.80 were observed for FCS48 and NCS starch samples. The observed increase in lightness (L*), and

Table 2. Effect of annealing on pH, TTA and color of the cassava starch samples.

Parameters	Samples					
	NCS	FCS24	FCS48	ANCS	AFCS24	AFCS48
pH	5.80^{a}±0.02	4.50^{c}±0.01	4.20^{e}±0.00	5.20^{b}±0.01	4.30^{d}±0.01	4.20^{e}±0.02
TTA(g/l)	0.14^{c}±0.02	0.24^{b}±0.01	0.38^{a}±0.02	0.14^{c}±0.00	0.24^{b}±0.02	0.39^{a}±0.02
L*	71.66^{f}±.0.12	82.31^{b}±1.13	79.41^{d}±0.40	73.71^{e}±0.14	83.55^{a}±0.39	81.13^{c}±0.97
a*	-4.93^{c}±0.21	-2.25^{b}±0.13	-1.29^{a}±0.12	-4.97^{c}±0.27	-2.10^{b}±0.32	-1.30^{a}±0.22
b*	6.80^{a}± 0.71	3.82^{e}± 0.02	5.51^{c}± 0.10	6.58^{b}± 0.17	2.81^{f}± 0.36	5.00^{d}± 0.31

Table 3. Effect of annealing and fermentation on the swelling power (%) of yellow cassava starch flour.

Temperature (°C)	Samples					
	NCS	FCS24	FCS48	ANCS	AFCS24	AFCS48
50	3.76^{a}±0.06	3.06^{d}±0.01	3.00^{c}±0.01	3.36^{b}±0.15	2.98±0.02	3.01±0.06
60	5.08^{a} ±0.01	3.88^{d}±0.02	4.01^{c}±0.01	4.11^{b}±0.02	3.75^{f}±0.02	3.83^{e}±0.01
70	11.67^{a}±0.0	6.93^{e}±0.03	8.86^{c}±0.03	9.05^{b}±0.01	6.84^{f}±0.02	8.56^{d}±0.01
80	16.04^{a}±0.01	14.65^{d}±0.03	13.86^{e}±0.05	15.11^{b}±0.01	13.10^{f}±0.01	15.03^{c}±0.02
90	18.11^{a}±0.10	14.65^{e}±0.03	17.45^{b}±0.02	16.05^{c}±0.02	14.10^{e}±0.01	14.52^{d}±0.03

Table 4. Effect of annealing and fermentation on the solubility (%) of fermented yellow cassava starch flour.

Temperature (°C)	Samples					
	NCS	FCS24	FCS48	ANCS	AFCS24	AFCS48
50	2.20^{f}±0.01	2.39^{e}±0.02	2.91^{c}±0.02	2.89^{d}±0.01	3.11^{b}±0.02	3.32^{a}±0.03
60	2.83^{e}±0.02	2.98^{d}±0.03	3.05^{c}±0.03	3.07^{c}±0.03	3.73^{b}±0.02	4.02^{a}±0.01
70	4.65^{f}±0.03	8.08^{b}±0.03	7.08^{c}±0.03	8.86^{a}±0.02	6.12^{e}±0.02	6.51^{d}±0.01
80	8.05^{f}±0.03	12.02^{a}±0.02	9.95^{d}±0.00	10.07^{c}±0.03	9.20^{e}±0.00	10.71^{b}±0.02
90	11.55^{e}±0.01	15.39^{a}±0.05	12.54^{c}±0.00	13.51^{b}±0.01	12.49^{d}±0.01	13.52^{b}±0.03

decrease in b* values in all the fermented and annealed samples could be due to decrease in the beta carotene content as a result of degradation of carotenoids which probably occured during the drying process and enzymatic oxidation by the oxidative enzymes may also have been activated when starch flour was mixed with water. (Burešová *et al*., 2023; Paznocht *et al*., 2019).

Effect of temperature on solubility and swelling power of the pro-vitamin A cassava starches

The results of the solubility and swelling power are presented in Tables 3 and 4. The treated (fermented and annealed) starch samples which swell differently at different temperatures and could indicate that the treatment and temperatures led to the reordering of the starch molecules. The physicochemical properties of starch are often modified by the annealing process, but the granular structure is unaffected because the annealing temperature is lower than the gelatinization temperature (Alvani *et al*., 2012). In this study, lower swelling capacity and higher solubility values were recorded for the fermented with or without annealing when compared with the native cassava starch (NCS). Slight increase in solubility and swelling was observed at 50-60°C while at 60-70°C, there was a drastic increase in all the samples. In overall, higher solubility was observed at 70°C and above. Generally, there was an increase in solubility and swelling as the temperature increases in starches fermented with or without annealing. This could be explained by the fact that as the temperature rises, there is more interaction between the branching segments of amylopectin in the crystalline regions and the amylose molecules in the bulk amorphous regions (Adebowale *et al*., 2002). Increase in solubility in the annealed starches could probably be due to the weathering of the starch granules during the annealing process hence, improved solubility. This report is line with the report of Adebowale *et al*. (2005) on red sorghum starches.

Conclusion

Fermentation rather than annealing improved the crude protein content of the starches while the combination of both fermentation and annealing resulted in improved ash and the β-carotene content when compared with the native yellow cassava starch in this study. However, the amylose content decreased in starches fermented with or without annealing irrespective of the fermentation period. Amylose leaching was more pronounced in the native cassava starch. The fermentation and annealing processes improved the solubility and the nutritional qualities in the treated vitamin A cassava starches and hence can be advantageous in bakery products.

DECLARATION OF COMPETING INTEREST

There is no conflict of interest or whatsoever from the authors.

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