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African Journal of Food Science

Full Length Research Paper

Physicochemical properties of starches extracted from local cassava varieties with the aid of crude pectolytic enzymes from *Saccharomyces cerevisiae* (ATCC 52712)

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Starches extracted from root mashes of the *Nkabom*, *Esam Bankye*, *Bankyehemaa*, *Doku Duade* and *Afisiafi* cassava varieties, with the aid of (crude) pectolytic enzymes from *Saccharomyces cerevisiae* pectolytic were analysed to compare effects of (pectolytic) enzyme technology on the physicochemical properties of the extracted starches. This was to help establish the extent to which varietal differences affect application of the technology and to inform the possible domestic or industrial application of the ensuing starches. Enzyme treatment generally did not affect the protein, fiber and ash content of the starches. However, it significantly increased moisture content, starch granule sizes, water binding capacity and swelling power of the starches in most the varieties; pH of the extruded starches were also significantly decreased and the starches' colour was also significantly made lighter by the technology. Despite the general trends observed, the technology was found to impact physicochemical properties of some varieties more than others. The work therefore showed that the technology is variety-sensitive and could influence starch utility.

Key words: Amylolysis, cassava varieties, crude pectolytic enzymes, endogenous amylase, starch physicochemical properties.

INTRODUCTION

In many parts of the world, enzyme application in industry has become common. The food, pharmaceutical, neutraceutical and medical sectors are all profoundly impacted by enzyme use. As of 2013, global market for enzymes was estimated at 4.8 billion dollars and demand is expected to rise to about 7.1 billion dollars in the year 2018; this comes to a compound annual growth of about 8.1% between the said periods (https://www.bccresearch.com/marketresearch/biotechnology/enzymes-industrial-applications-bio030h.html).

In many parts of Africa, enzyme technology is a rather nascent novelty; it is applied, in some cases, in total oblivion in traditional food processing (such as in the fermentation of cassava chips and dough to produce

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foods like *kokonte* and *agbelima* respectively). Biocatalysts which serve as a source of enzymes for such (fermentation) processes are applied either submerged in a (nutrient) medium or incubated on a solid substrate. Very little documentation is however available on how such processing methods impact the physicochemical properties of the resulting food substances. Starch, like enzymes, is an important commodity whose industrial applications are diverse - ranging from the adhesive and textile to food and medical sectors. In Ghana, starch is recovered from root crops such as yam, cassava and potatoes. Cassava is however the crop of choice for starch production and different varieties are available for dietary and industrial considerations. This presents a challenge with regards to utility as certain varieties could be heavily depended on because of their appealing qualities for food or their inherent starch yield. The *Afisiafi* cassava variety, for example, has been cited as good for both starch production and for indigenous foods like *kokonte* (dried fermented chips), *agbelima* (tubers milled into dough) and *gari* (grated, fermented sieved and fried mash) (http://rtimpknowledgecenter.blogspot.com/2014/07/improved-cassava-varieties.html).

One of the reasons cited for the collapse of the Ayensu Starch Factory, set up in 2008 as part of the Ghana governments Presidential Special Initiative (PSI) on cassava, was the lack of raw materials needed to feed the factory. Attempts at recovering starch using enzyme technology have achieved remarkable results, boosting yields and impacting positively the utility of the resulting starches. However, this feat (in many cases) has only been achieved at the lab scale with single crop varieties. Response of variety to effects of the technology on the physicochemical properties of the ensuing starches is therefore poorly studied. Previous work using crude pectolytic enzyme preparations showed some amylase activity (Agyepong and Barimah, 2017). However, as starch granules from different varieties of the same crop species show some differences in granule morphology and chemistry (including starch and amylose content), it was expected that the technology will impact the resulting starches differently. Effects of the (enzyme) technology on starches from the various cassava varieties will help inform the utility of the ensuing starches as yields are also enhanced.

MATERIALS AND METHODS

Plant materials and chemicals

Root tubers of fresh local cassava (*Manihot esculenta* Cranz) varieties *Afisiafi*, *Esam bankye*, *Bankyehemaa*, *Nkabom* and *Doku duade* harvested at 9 months after planting (MAP) were obtained under a running project at the Department of Agriculture Engineering, K.N.U.S.T. (Kumasi, Ghana). All varieties were planted on the same field of the Agriculture Research Station (at *Anwomaso-Domeabra*, Kumasi) and had been subjected to similar edaphic and climatic conditions.

All chemicals used for the project were (analytical grade) products of SIGMA® and FISON ®. These were obtained from the Labs of the Departments of Biochemistry and Biotechnology and Theoretical and Applied Biology, KNUST.

The microbe used for enzyme production was yeast (*Saccharomyces cerevisiae* ATCC 52712). This strain of yeast, purchased from America Type Culture Collection, Maryland, USA, had been maintained on agar slant at the Department of Biochemistry and Biotechnology, KNUST, Kumasi.

Cell culture and enzyme production

Prior to enzyme production, *S. cerevisiae* (ATCC 52712) cells were propagated and subcultured in malt extract broth (M.E.B.) and malt extract agar (M.E.A.) slants to obtain pure cultures. A loop full of pure culture from M.E.A. slant was inoculated in 100 ml malt extract broth and incubated for 3 days at 28°C. During the period, light absorbance (at 540 nm) and cell enumeration (on malt extract agar using pour plate technique) were taken at 12 h intervals. The values obtained were used to derive a standard calibration curve for cell density.

Production and assaying of crude pectolytic enzymes

Four milliliters (4 ml) of M.E.B. culture (cell density 6.32×10^{12} per 100 ml) was subcultured in 100 ml of 1% pectin medium (formulated based on a modification of the method used by Ranganna (1986)) for 8 days at 28°C to induce carbon catabolite repression and to stimulate the production of yeast pectolytic enzymes in the medium. During this period, the concentration of crude protein (enzyme) was monitored (using the Biuret test) and cell density was estimated daily at 540 nm using spectrophotometry vis-à-vis the standard (calibration) plots for cells density obtained; crude protein (enzymes) were obtained by centrifugation at a speed of 3600 g for 10 min at a temperature held at 4°C.

Extracts from the 1% pectin medium were also monitored (during the 8 day incubation period) for their pectolytic activity (Jayani et al., 2005). One unit of polygalacturonase (PGase) activity was as defined by Jayani et al. (2005).

Endogenous amylase enzyme assay

Crude pectolytic enzyme preparations have been reported (Dzogbefia et al., 2008a) to contain some amylase activity. Hence, over the 8-day period of incubation, amylase activity in crude enzyme extract was assayed with laboratory grade starch (BDH). This was based on modification of the method described by Bernfeld (1955).

1% starch solution was prepared by dissolving 1 g starch in 100 ml of slightly warmed sodium acetate buffer (0.1 M, pH 4.7). The extraction buffer was 1 M potassium hydrogen phosphate, pH 6.5. One milliliter (1 ml) of 1% starch and 1 ml of the crude enzyme extract were incubated at 27°C for 15 min. At the end of the incubation period, the reaction was stopped by the addition of 2 ml of dinitrosalicylic acid reagent and the resulting solution heated in a boiling water bath for 5 min. While the test tubes and its content was warm, 1 ml of 40% potassium sodium tartrate solution was added and the content cooled in running tap water. The volume was then made up to 10 ml by the addition of 6 ml water. The absorbance was read at 560 nm. In the case of the control, the reaction between the 1% starch and crude enzyme extracted was terminated at zero time. The amount of the reducing sugars formed was calculated from a standard graph prepared from known concentrations (10-100 mg) of maltose.

Preparation of cassava mash for starch extraction

Freshly harvested cassava varieties (*Afisiafi*, *Doku duade*, *Esam bankye*, *Nkabom* and *Bankye hemaa* all harvested at 9 months after planting, MAP) were each sorted, washed under running tap water and knife peeled. Moisture content of the pulp from the cassava varieties was determined using the AOAC Method (1990). The peeled cassava was washed in distilled water and cut into 2-3 cm^3 chunks. 100 g of diced cassava pulp from each variety (in triplicates) were selected and frozen after which they were blended separately in a double screw waring blender (model 32 BL80 (8011), USA) set at low speed (18000 RPM) for 1 min. The chilling treatment was to minimize starch gelatinization when blending and the low rotor speed was to minimize granule shearing as this could influence enzyme activity, granule structure and other starch physicochemical properties. Cassava mash of each variety was transferred to into 600-ml conical flask and labeled accordingly. 100 ml of distilled water was added to each of the samples and shaken to dissolve the ensuing mash.

Stages for the enzyme-based starch extraction are as shown in the flow chart (Figure 1). Starches obtained from optimum treatment combinations (retention time versus crude protein dosage) for yield were sampled and effects of enzyme treatment on their physicochemical parameters were investigated. Optimum starch yield was recorded with the 0.2% crude protein at 0.5 h (retention time) in the *Nkabom* and *Esam Bankye*; *Afisiafi* required similar crude protein dosage but at 1 h retention time. *Doku duade* and *Bankye hemaa* both recorded yield optima with the 0.25% dosage at 0.5 h retention time (Agyepong and Barimah, 2017).

Microscopic observation of starch granules

Starches from controls and treated samples of all varieties were mounted in distilled water and observed under binocular compound microscope. Observation was done at high power (X 400). For starch granule diameter measurements, a micrometer disk (eyepiece graticule) and a stage micrometer were used. Starch granules from each (control) variety and for those obtained from their corresponding (enzyme) treatments, were categorized as *Small*, *Medium* and *Large* based on gross visual inspection. Thereafter, their corresponding measurements were carried out by selecting randomly three granules from each category and the mean size determined.

Determination of starch pH

The extracted cassava starch (10 g) was weighed and made into slurry using 25 ml of distilled water. The pH of the slurry was determined using the Corning pH meter (model 240). This was done for both control and enzyme treated samples.

Determination of starch moisture content

Two grams (2 g) of samples were weighed into previously dried and weighed glass crucibles. The crucibles with the samples were then placed in thermostatically controlled oven (XOV 880, Gallenkamp, England) at 105°C for 5 h to obtain starch dry mass. At the end of the period, the crucibles were removed and placed in a desiccator to cool, and their weights recorded till a constant weight was obtained. Percentage moisture contents of the starches were calculated (AOAC, 1990).

Determination of ash content of starch

Ash content of the starch samples was determined by the Dry Ashing Method (AOAC, 1990). Two grams (2 g) of samples was weighed into previously ignited and weighed porcelain crucibles. The crucibles and their contents were then placed in a Muffle furnace (Model AS 260D, Gallenkamp, England) preheated to 600°C and heated for 2 h. The ash content in each was calculated and expressed as a percentage.

Determination of crude fiber content of starch

Crude fiber content of samples was determined by the AOAC (1990) method.

Determination of starch protein content of starch

The Association of Official Analytical Chemists (AOAC, 1990) Kjeldahl procedure was used to determine the crude protein content of starch samples.

Determination of amylose content of starch samples

Amylose content of the starch samples was determined based on the iodine colorimetric method described by McCready and Hassid (1943). One hundred milligrams (100 mg) each of cassava starch samples was introduced into 100 ml volumetric flask, wetted with 1 ml ethanol and 10 ml distilled water. The content was dissolved by adding 2 ml of 10% NaOH, and heated in water bath to form a clear solution. The flask with its content was cooled and diluted to the mark. Five milliliters (5 ml) portion (equivalent to 5 mg) of the alkaline starch solution was introduced into a 500-ml volumetric flask; 100 ml of water added and slightly acidified with 3 drops of HCl. The contents were well mixed by shaking the flask and 5 ml iodine was added to the mixture and diluted to 500 ml with distilled water. A blank was set by diluting 5 ml iodine solution to 500 ml with distilled water in place of the standard sample. The absorbance of each starch sample was read against the blank in the spectrophotometer set at 640 nm. The percentage amylose of samples was determined using an equation derived from a standard calibration curve for amylose.

Determination of water binding capacity

Water binding capacity of starch samples was determined following the method of Yamazaki (1953) as modified by Medcalf and Gilles (1965). An aqueous suspension was made by dissolving 2 g of cassava starch in 40 ml distilled water in a previously dried and weighed centrifuge tube. The suspension was agitated for 1 h on a Griffin shaker and centrifuged at 2200 rpm for 10 min. The centrifuge tube was inverted for 10 min to drain off unbound water. The centrifuge together with its content was weighed and bound water was calculated and expressed as a percentage.

Determination of solubility and swelling power

Solubility and swelling power of the starch samples were determined based on the modified method of Leach et al. (1959). One gram (1 g) of cassava starch sample was dissolved in 40 ml distilled water in a previously weighed 50-ml centrifuge tube. The suspension was stirred just sufficiently and uniformly avoiding excessive speed to prevent starch granule fracture. The suspension was heated at 85°C in a thermostatically regulated water bath for 30 min with constant stirring. It was then centrifuged at 2200 rpm for 15 min. The solubility was found from the residue after evaporating the supernatant in a previously dried and weighed glass crucible.

Figure 1. Stages involved in the processing of cassava tubers for enzyme-aided starch extraction.
Source: Agyepong and Barimah (2017).

The percent solubility and swelling power were then determined.

Determination of starch colour

The colour of samples of starch from both treated and untreated mashes of each variety were examined using a colour meter (Minota, chromameter CR-2001). The L×a×b* (Chroma meter) colour system was used to calibrate a white tile.

Statistical tool(s) and analyses

For all parameters measured, statistical analyses were carried out

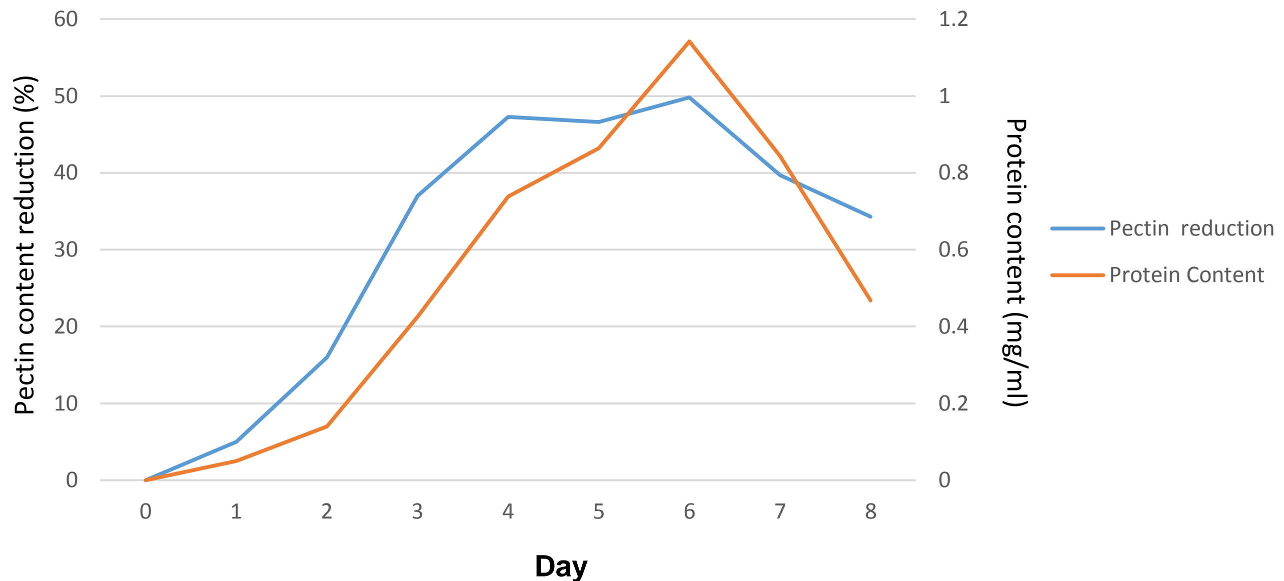


Figure 2. Protein content and pectolytic activity of crude protein extracts during incubation with *S. cerevisiae* (ATCC 52712).

with SigmaPlot for Windows Version 11.0 by Systat Software Inc. © 2008. Graphs were plotted using Microsoft Excel 2007 (from Microsoft Inc.).

The Completely Randomized Design (CRD) was used to organize all data. Data were subjected to a one-way ANOVA (unless otherwise stated) and significant differences were tested using the Duncan's New Multiple Range Test. All results were presented as mean ± standard values of three replicates (n=3) and Least Significant Difference (LSD) were also determined for all parameters. Significant differences between treatment means were accepted at $P<0.05$ (unless otherwise stated).

RESULTS AND DISCUSSION

Pectolytic and amylase activities in crude enzyme extracts

On the 6th day of culturing *S. cerevisiae* in the 1% Pectin medium (Figure 2), the resulting extract recorded its highest protein content and pectolytic activity of 4.91 U (with specific activity 4.21 U/mg) and a 0.293 U/ml (with a specific activity of 0.257 U/mg) of endogenous amylase activity in the extract.

Starch granule size and structure

Enzyme treatment enhanced the sizes of medium to large starch granules extracted from all varieties. However, sizes of small starch granules extracted from most varieties were not improved by the technology (Table 1).

Photomicrographs of starch granules from root (pulp) mashes of untreated and treated cassava varieties are presented in Plates 1 to 5.

Starch granule size affects starch composition and other functional properties such as gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling and solubility (although several other factors, including amylose/amylopectin ratio and molecular weight and granule fine structure, are also influential) (Lindeboom et al., 2004). An improvement in the recovery of starch granule size (as a result of enzyme treatment) suggests a general enhancement in digestibility and in its performance as a binding agent (Sandhan et al., 2017). Starch granule sizes obtained ranged from 5.33 - 23.34 µm (Table 1). This range of values agrees with the 4 - 35 µm range reported by Adejumo et al. (2011). Dzogbefia et al. (2008b) recorded similar granule sizes for the various categories of starches they extruded from untreated *Afisiafi* (aged 6MAP). This confirms reports (Moorthy and Ramanujam, 1986) that the granule size of starch does not increase significantly after the 6th M.A.P. However, larger starch granules were observed in our treated samples for the same variety. This observation could be due to the differences in enzyme activity on the *Afisiafi* cassava mash. Thus, the crude enzyme used for this work, probably had slightly varied composition of pectolytic enzymes that was more effective at hydrolyzing pectins in the mash thus liberating much bigger starch granules that could have otherwise been trapped within the fiber matrix.

On morphology of all (non-fractured) granules, the photomicrographs (Plates 1 to 5) showed near-spherical starch granules, each with three shallow equidistant surface fissures that radiate from a deep central groove towards the edge of the granule; the grooves diminish as they approach the edge of the granules. Yuan et al. (2007) confirmed the presence of incomplete hemisphere

Table 1. Sizes of starch granules extracted from the treated and untreated cassava root mashes.

Cassava variety		Categories of starch granules and their average diameter (µm)			
		Small	Medium	Large	Avg. size
Nkabom	Control	6.67±1.9	13.34±0.0	18.67±0.0	12.89
	Treated	**6.67±1.9**	**13.34±0.0**	**20.01±1.9**	**13.34**
Afisiafi	Control	6.67±1.9	13.34±0.0	18.67±0.0	12.89
	Treated	**6.67±1.9**	**12.94±0.6**	**21.34±0.0**	**13.65**
Bankye hemaa	Control	9.34±1.9	13.34±0.0	21.34±3.8	14.67
	Treated	**9.34±1.9**	**14.67±1.9**	**23.34±0.9**	**15.78**
Esam Bankye	Control	5.33±0.0	14.67±1.9	20.01±1.9	13.34
	Treated	**7.34±0.9**	**18.34±0.0**	**22.67±1.9**	**16.12**
Doku duade	Control	7.33 ± 0.9	13.34±0.0	20.01±1.9	13.56
	Treated	**4.67 ± 0.9**	**13.34±0.0**	**23.34±1.9**	**13.12**

Values are represented as mean ± standard deviation (n=3); n = number of replicate mashes.

granules with fissures on tapioca starch. Starch granule size, shape and their surface characteristics are important in characterizing and identifying the botanical source of starch (Robertson et al., 2006).

From the photomicrographs (Plates 1 to 5), it is clear that generally, enzyme treatment did not have much detrimental effect on starch granule structure. However, it is observed that starches from the *Bankyehamaa* and *Nkabom* (Plates 1 and 4 respectively) recorded fractures in some granules from both the treated and untreated mashes. These fractures result from effects of processing mechanisms such as maceration, freezing of mashes during storage (Sujka and Jamroz, 2007) and solar drying of granules (Huber and BeMiller, 1997). According to Sujka and Jamroz (2007), freezing starches of different water content causes granule surface crushing and destroys granule inner structure (acting in a way similar to high pressure).

Fractures observed, especially in the central grooves of starch granules from the treated *Doku duade* and *Esam bankye* (Plates 2 and 3 respectively) varieties, suggest that enzyme treatment contributed to granule fracture in those regions. There are reports (Sujka and Jamroz, 2007) that the presence of pores, channels and cavities on the surface of starch increases the surfaces potentially available for chemical and enzymatic reactions. Thus, activities of endogenous amylases were probably high in these grooves and micropores (which cannot be resolved by conventional light microscopy) making the granules more vulnerable to fracture in these areas. The effects, however, might have been minimized by the shorter retention times adopted for mash incubation (since not all treated granules showed the detrimental effects) during enzyme treatment and starch extraction.

Thus effects of enzyme treatment on starch granule structure of different cassava varieties varied. The technology rendered some granules more susceptible to fragmentation whereas others were not significantly affected.

Moisture content and pH

Starch samples from all enzyme-treated mashes had higher moisture content than their respective controls (Table 2). As moisture content correlates negatively with amylose content (QinKe-xin et al., 2014), it is possible that reduction in amylose-water interaction (due to amylolysis) might have caused this. Moisture content of starches from both treated and untreated varieties differed significantly but agreed with values reported in literature (Oladunmoye et al., 2014; Belibi et al., 2014). Higher than reported moisture content reduces the shelf life of the starch as it tends to enhance the growth of moulds which subsequently affects other important qualities like its colour, protein and amylose contents.

Moisture content of starches from different cassava varieties can be affected by a number of factors which include intensity and duration of thermal exposure, prevailing ambient humidity (Apea-Bah et al., 2011) as well as aeration: morphological features of the starches' microstructure could also influence water sorption.

Enzyme treatment also reduced the pH of the ensuing starches. Acetate buffer used for enzyme production (for cassava mash incubation) might have influenced this outcome. Starch pH ranged from 5.2 – 7.6 (the lowest pH being recorded in the *Doku duade* and the highest from the *Esam bankye* varieties). However, these do not

Table 2. Physicochemical properties of starches from the various cassava varieties before and after enzyme treatment.

Physicochemical Parameter	Cassava varieties									
	Nkabom		*Esam Bankye*		*Bankyehemaa*		*Afisiafi*		*D. duade*	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
pH	6.12[a] (0.01)	6.03[b] (0.00)	7.86[c] (0.00)	7.62[d] (0.02)	7.19[e] (0.02)	7.04[f] (0.00)	6.92[g] (0.00)	6.81[h] (0.01)	6.29[i] (0.01)	6.22[j] (0.02)
Moisture (%)	8.21[a] (0.29)	9.32[b] (0.25)	11.69[c] (0.58)	12.39[d] (0.12)	10.26[e] (0.43)	10.47[f] (0.41)	10.72[g] (0.34)	11.05[h] (0.17)	12.07[c] (0.44)	12.10[c] (0.46)
Total ash (%)	0.98[a] (0.01)	0.96[a] (0.01)	0.50[b] (0.01)	0.49[b] (0.01)	0.38[c] (0.01)	0.39[c] (0.01)	0.49[b] (0.01)	0.49[b] (0.01)	0.50[b] (0.01)	0.50[b] (0.01)
Crude Protein (%)	0.09[a] (0.00)	0.09[a] (0.00)	0.04[b] (0.00)	0.04[b] (0.00)	0.03[c] (0.00)	0.03[c] (0.00)	0.06[d] (0.00)	0.06[d] (0.00)	0.09[a] (0.00)	0.09[a] (0.00)
Crude Fiber(%)	0.15[a] (0.08)	0.14[b] (0.05)	0.12[c] (0.05)	0.12[c] (0.07)	0.12[c] (0.06)	0.12[c] (0.04)	0.13[d] (0.07)	0.12[c] (0.03)	0.14[e] (0.04)	0.14[e] (0.06)
Solubility (%)	4.81[a] (0.37)	22.66[b] (0.18)	1.29[c] (0.01)	4.14[a] (0.07)	2.00[c] (0.02)	19.08[d] (0.08)	7.38[e] (2.21)	8.00[e] (0.12)	1.70[c] (0.03)	22.08[b] (0.05)
Amylose (%)	23.79[a] (0.17)	20.57[b] (0.05)	36.33[c] (0.20)	29.29[d] (0.80)	42.47[e] (0.44)	41.47[f] (0.41)	27.40[g] (1.11)	19.71[b] (0.35)	48.79[h] (0.68)	41.09[f] (0.20)
Water Binding Capacity (%)	52.80[a] (0.51)	62.57[b] (0.09)	66.56[c] (0.03)	66.91[c] (0.27)	71.91[d] (0.09)	77.93[e] (1.53)	61.86[b] (2.32)	80.03[f] (0.57)	69.88[g] (0.30)	65.40[c] (0.53)
Swelling Power (%)	13.31[a] (0.12)	13.49[a] (0.11)	8.62[b] (0.09)	13.29[a] (0.13)	10.58[c] (0.02)	17.15[d] (0.26)	14.47[e] (0.05)	14.15[f] (0.15)	6.41[g] (0.47)	10.70[c] (0.41)

a) Values in parenthesis () are standard deviations of mean (triplicate) determinations
a) Means in a row followed by the same letter are not significantly different (P>0.05)

deviate from starch pH values reported in literature (Garrido et al., 2014).

Protein, fiber and ash content

Enzyme treatment did not affect the protein content of the ensuing starch (Table 2) but recorded values were lower than reported (Aldana and Quintero, 2013). High protein content negatively affects starch pasting properties (Iwe et al., 2017) due to the reduction in hydroxyl groups caused by Maillard reactions. Hydroxyl groups in starch are key physicochemical determinants of starches (Silvaa et al., 2017).

Ash content was not significantly affected by enzyme treatment (Table 2) and the values recorded agreed with those in literature (Adjei et al., 2017). Although cultivated in the same soil having similar edaphic features and chemistry, there were significant differences (P<0.05) in ash content between varieties suggesting that mineral uptake by cassava root parenchyma differ significantly between varieties.

Although starches from some of the treated mashes of the *Afisiafi* and *Nkabom* showed significant reduction in crude fibre content, this parameter was significantly (P<0.05) not affected by enzyme treatment in the other varieties. Fibre content was generally lower than reported in literature for cassava starch (Dzogbefia et al., 2008a).

Effects of enzyme treatment on amylose content

Amylose content of starches from the *Afisiafi* and *Nkabom* varieties agreed with values for cassava starch cited in some literature (Ojo et al., 2017; Rolland-Sabaté et al., 2012), whereas those from *Bankye hemaa*, *Esam bankye* and *Doku duade* did not. Amylose content of flour from some cassava varieties in Ghana has been reported to range between 10.9 - 44.3% (Aryee et al., 2006). Despite these disparities our primary objective of investigating the effect of the technology, from (percentage) changes in their physicochemical

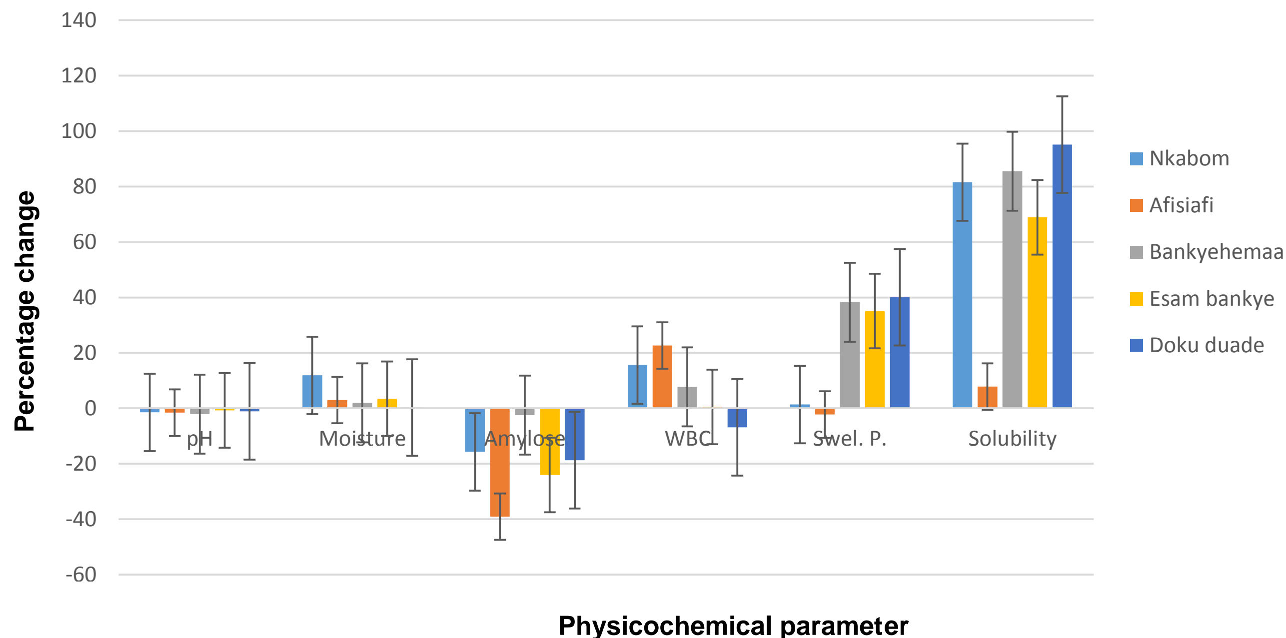


Figure 3. Physicochemical parameters of starches affected by enzyme treatment.

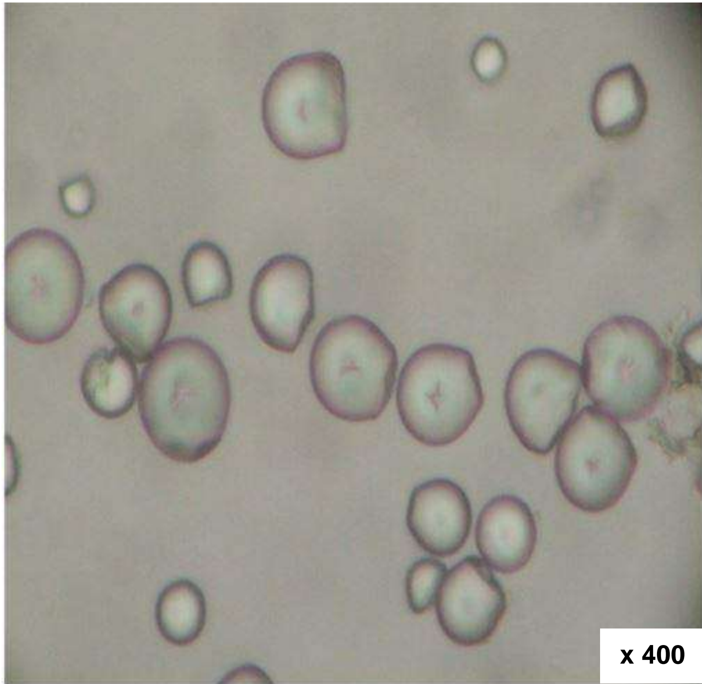
characteristics (Figure 3), was possible since starches from both control and treated mashes were subjected to the same experimental conditions and protocols.

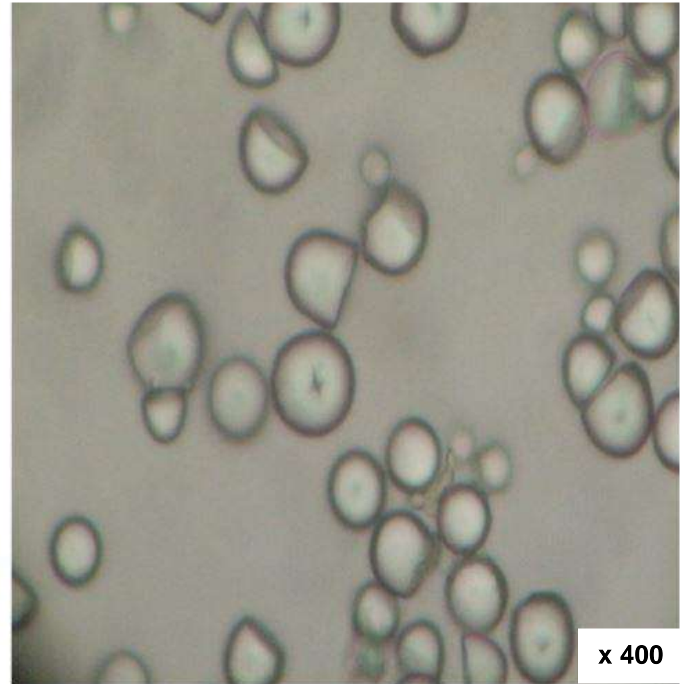
Starches from treated root mashes of all varieties showed significant reduction in amylose (Table 2). High amylase activity recorded in the crude enzyme would explain the generally high amylolytic effects. The very high degradation of amylose in the *Afisiafi* variety could be attributed to the longer period of exposure of its (pulp) mashes to the crude enzymes (1 h incubation time compared with 30 min in the other varieties). Since the enzyme was from a crude source, overall amylase activity would depend on the type and quantity of amylase enzymes present (Bijttebier et al., 2008); type of ions present (which could serve as inhibitors and/or cofactors to amylase and pectinases) in the mash (Bijttebier et al., 2008; Singh et al., 2014) and differences in (amylose) molecular architecture (starch crystalinity), which will determine availability of amylose for amylase action (Polesi et al., 2017). This latter feature (of starch) is variety dependent (Alcázar-Alay and Meireles, 2015).

Effects of amylase on most starches have also been linked with starch granule sizes. Qi and Tester (2016) have reported that small to medium granule starches are more susceptible to enzymatic degradation due to the higher surface area they present. Thus the relative proportions of a particular category of starch granules released (by pectolytic activities) would greatly influence the observed amylolytic effects in the variety: thus interplay of the two groups of enzymes influences the quality of the starches extracted. Varieties that yield more of the larger granules from their mash will be less susceptible to amylolytic effects of the technology. This probably explains why the *Bankyehemaa* variety was more resilient (Figure 3) to amylolysis as the variety recorded the largest granular sizes from both its untreated and treated mashes (Table 1). As high amylose content negatively correlates with digestibility (Fouhse et al., 2015), enzyme application in high amylose/small granule size varieties will yield starches that will be greatly enhanced for application in the food industry. Thus, starches from the *Doku duade* and *Esam bankye*, high amylose varieties (Table 2) which recorded high amylolysis (Figure 3) at short incubation time of 30 min, will be greatly improved for food application if their incubation time with enzymes was extended.

Effects of enzyme treatment on swelling and solubility index

All the starches swelled when heated to a temperature of 80°C. As amylose correlates negatively with swelling power and solubility (Yu et al., 2015), it was expected that the swelling power of high amylose varieties (*Bankyehemaa*, *Doku duade* and *Esam bankye*) would be low (Table 2). Amylose is responsible for maintaining associative forces within granules (Valcárcel-Yamani et al., 2013) hence starches from high amylose varieties would have stronger associative forces that resist swelling upon heating. Reduced swelling also reduces the freeness with which soluble products leach out of

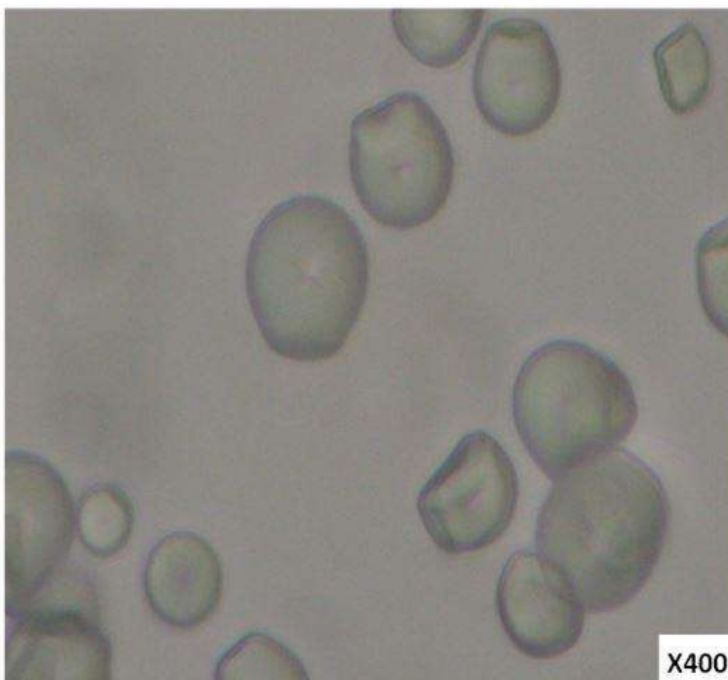


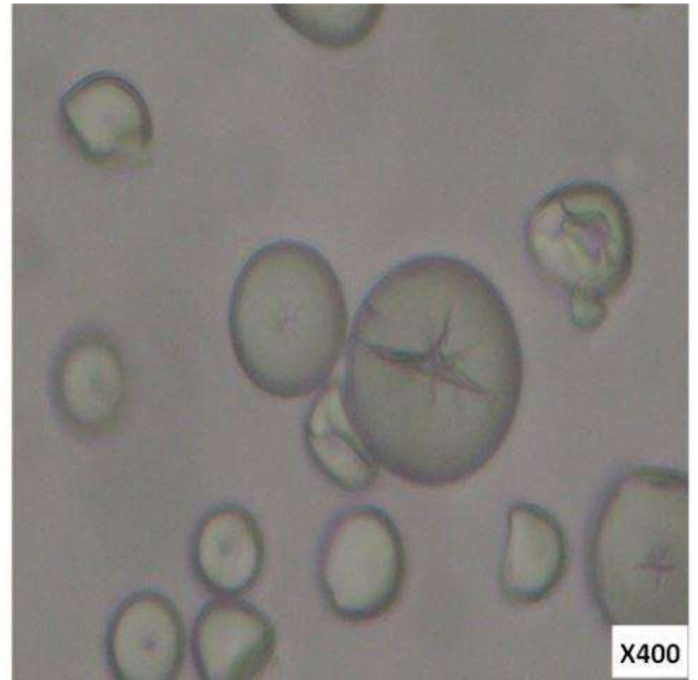


Starch granules from the untreated (Control) mash

Starch granules from the treated mash

Plate 1. Granule structure of starches obtained from untreated (control) and enzyme-treated cassava mash from the *Bankye hemaa* variety.

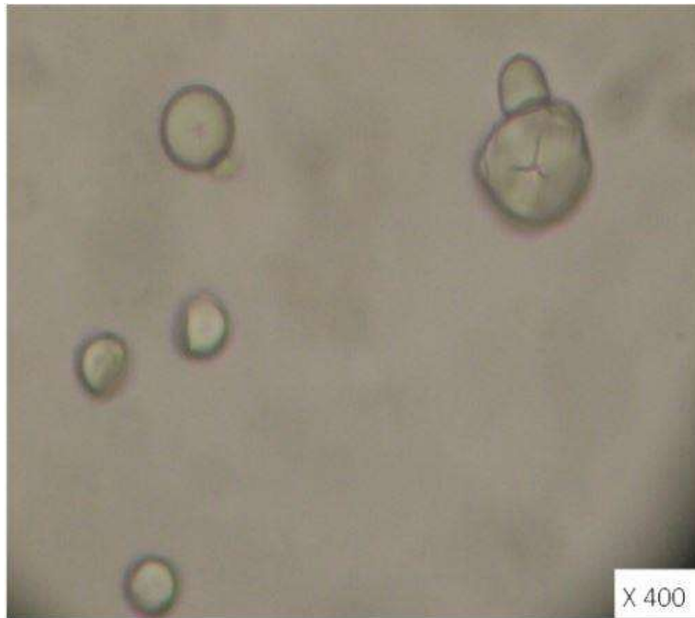




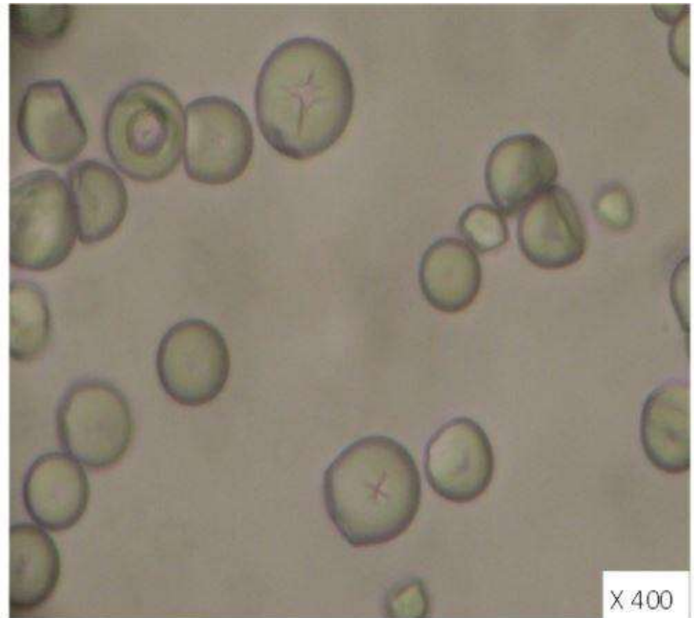
Starch granules from the untreated (Control) mash

Starch granules from the treated mash

Plate 2. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Doku duade* cassava variety.

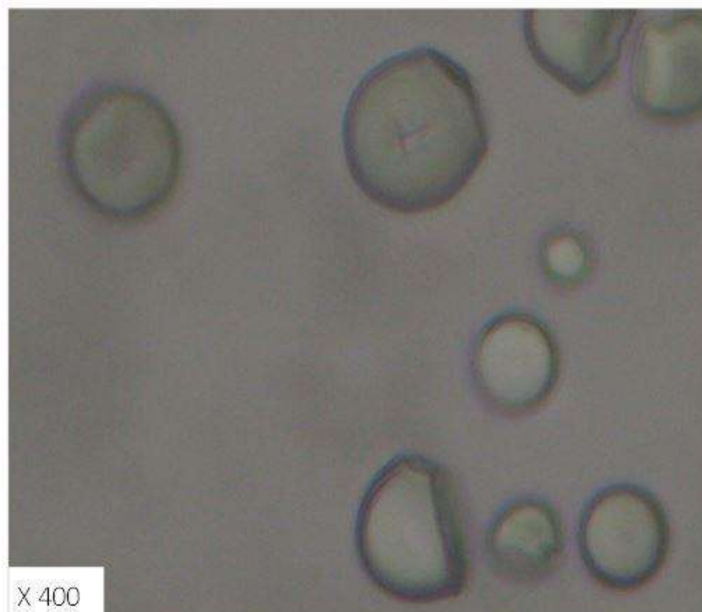


Starch granules from the untreated (Control) mash



Starch granules from the treated mash

Plate 3. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Esam bankye* variety.

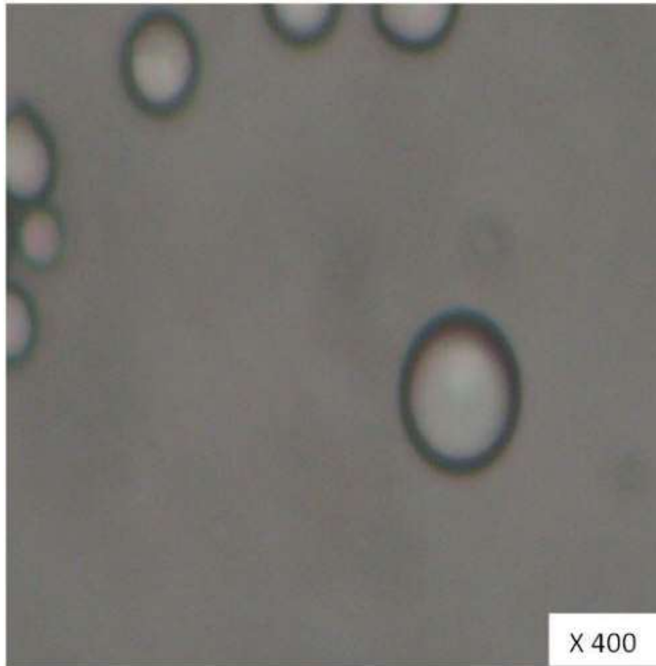


Starch samples from the untreated (Control) mash

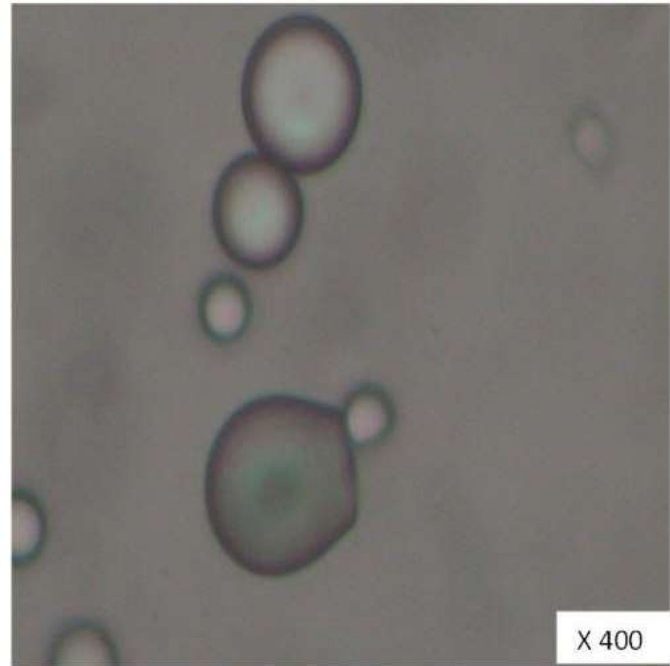
Starch samples from the treated mash

Plate 4. Granule structure of starches extracted from untreated (control) and enzyme – treated cassava mash from the *Nkabom* variety.

their granules. Starches from the treated samples swell better than those from their respective control samples (Table 2). Reduction in amylose content of starches from the treated mashes, due to activity of endogenous amylase, reduced amylose-amylose interaction (via hydrogen bonding) in the granules. This also meant that enzyme treatment reduced crystallinity of the starch granules allowing the granules to leach out more amylose



Starch samples from the untreated (Control) mash



Starch samples from the treated mash

Plate 5. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Afisiafi* variety.

to associate with water. Apparently, starches from varieties whose amylose content were least affected by enzyme treatment could swell better than those from their respective non-treated mashes (Figure 3). Also, swelling association with water. Starches inherently low amylose swell better; however when their amylose content was further compromised, their ability to swell further reduced. This explains the failure of starches from treated *Afisiafi* variety to swell more than its control. The extended incubation time required to peak starch recovery from the variety had detrimental effects on its amylose content. The lowered solubility products recorded in starches from the same variety could be due to its inability to retain (soluble) products as these were probably lost to the supernatant during stages of starch washing and decanting (Figure 1). Starches from all controls samples, especially those from high amylose (cassava) varieties, recorded low solubility values (Table 2) due to their granular thermostability (Omojola et al., 2010).

The relative abundance of the category of starch granules (*Small, Medium* or *Large*) released might also have influenced the solubility pattern observed. It is obvious that the release of small sized granules (Table 1) by enzyme treatment enhanced solubility values (Table 2). Qi and Tester (2016) have reported that small size starch granules tend to leak out more amylose out of their intact granule than do larger ones at 55°C and higher. Thus, smaller sized granules have higher solubility at temperatures above 55°C. The additive effects of smaller power of starches from the treated *Nkabom* variety was not significantly (P>0.05) affected probably as a result of its ability to resist amylolysis. extensive loss of amylose from low amylose starches drastically reduced amylose granule sizes in providing larger surface area for amylase action (Qi and Tester, 2016) could greatly increase the solubility of such starches. Thus, although high amylose containing varieties (*Doku duade*, *Bankyehemaa* and *Esam bankye*) provided enough substrate for amylolysis and subsequent solubilization; the same varieties (especially the *Doku duade* and *Esam bankye*) also recorded the least of the granule sizes (Table 1) further providing a larger surface area for enzyme action and rendering them more susceptible to degradation.

The high susceptibility of *Doku duade* starch granules to fragmentation (Plate 2) also explains its exceptionally high swelling power and solubility (Figure 3). The adhesive industry would find amylolytic effects of endogenous amylase undesirable as amylose degradation affects amylose-amylopectin ratio which is an important factor in determining the adhesive strength of starches (Gadhave et al., 2017). Hence, enzyme application aimed at enhancing starch yield for the adhesive industry would require the incorporation of amylase inhibitors, such as maltodextrins and acarbose (Robyt, 2005), to mitigate amylase degradative effects and help to maintain starch granule integrity. However, if the starch is to be applied to food, then such inhibitors could be eliminated as amylases enhance starch

digestibility (Cruz et al., 2015).

Effects of enzyme treatment on water binding capacity (WBC)

Enzyme treatment had varying effects on water binding capacity of the starches produced suggesting some varietal-sensitivity. Enzyme treatment enhanced WBC in the *Bankyehemaa* and *Nkabom* while the starches from the *Esam bankye* were not affected (Table 2). However, WBC for the *Doku duade* and *Afisiafi* recorded significant (P<0.05) reduction.

Water binding capacity is a function of granule size and amylose/amylopectin ratio. The treated *Doku duade*, releasing the smallest size starch granules (Table 1) with high amylose content (Table 2), suggests that a unit mass of starch from treated *Doku duade* would present a much larger surface area to volume ratio for its amylose to adsorb water. Its high amylose content (Table 2) and fractured granule morphology (Plate 2) also suggest greater susceptibility to amylolytic attack which subsequently reduces the associative hydrogen and covalent forces in the starch (Polesi et al., 2017). This might also have affected its bulk density (resulting from reduction in the amylose per granule).

Enzyme treatment best enhanced water binding capacity in starches from the treated *Afisiafi*. The long (enzyme) incubation time (1 h) required for peak starch yield in the variety clearly contributed to an enhancement in WBC as amylolysis enhances granule association with water. However the relative abundance of the type/category of granule could also have contributed to this observation. Such improvements in WBC (due to application of the enzyme technology) suggest that the technology, if adopted, would enhance the quality of the starches as drug binders and disintegrants in pharmaceuticals (Adjei et al., 2017). In the food industry, it could also be applied to ketchups to further enhance the stability and prevent separation of water in the food product.

Effects of enzyme treatment on starch colour

A three-way analysis of variance (with interaction) carried out on L×a×b parameters of the starches' colour indicated differences (P<0.5) and strong interaction (P<0.01) between variety and effects of enzyme treatment on starch lightness (*L* values), red to greenness (*a* values) and blue to yellowness (*b* values) (Table 3). The sense (positive or negative) of the (L×a×b) values was also not affected for all the varieties, showing negative values (redness) for the *a* and positive values for the *L* and *b* (whiteness and blueness respectively) (Table 3).

Significant difference in starch lightness between the varieties could be due to the differences in the type and amounts of proteins and the associated chromogenic compounds (including mineral ions) present in the starch. Studies by Rojas et al. (2007) on mineral absorption pattern of the cassava root revealed that the roots absorb high amounts of K, P, Zn, Br, Cl, S, B and Rb ions. Other well represented ions were from elements of Ca, Mg, Ba, Sr, Cu, Au and Ni and those under represented were those from Na, Fe, Si, Sn, Mo, Cd and the heavy metals like Pb and Hg. Highly soluble salts of these elements, could remain strongly bound to starch granules (at low concentrations after treatment and processing of the tuber) influencing ash content, solubility and colour. Individual or combined interactions of these elements and pigments with white light could have produced the characteristic yellow or blue tints observed in the starches.

All treated samples, for each variety, were significantly lighter compared with their controls (Table 3). Pectinases have been reported to improve the lightness of many processed plant products including starch (Hebeish et al., 2010). At low pH, mineral sorption to cassava starch tends to decrease due to hydrogen ions strongly competing with (mineral) adsorbates (Shah et al., 2015). Hence enzyme treatment might have reduced the amounts of mineral ions in the starches extruded; however this did not seem to have significantly affected ash content (Table 2).

Differences in starch colour observed are also likely to be influenced by the presence of soluble chromogens in the pulp of the cassava parenchyma (Carvalho et al., 2004). Dominant among such compounds are members of the carotenoid group which are known to impart yellow and red colour to the tissues.

White starches are better patronized than dull coloured ones (Lestari et al., 2012) probably because of its contribution to the aesthetics of the final product. Therefore, use of enzyme technology apart from enhancing starch usability, also enhances starch (as well starch-base products) appeal for patronage by the consumer.

Conclusion

Activities of crude pectinases greatly enhanced the extraction of large starch granules from their respective mashes. However, surface morphology of starch granules from some varieties seem to compromise structural integrity (upon treatment) as these granules were more susceptible to fragmentation by endogenous amylases in the crude enzyme preparation. Presence of endogenous amylase in the crude extract generally decreased amylose content; the extent of decrease was however also dependent on starch granule size and amylose content of starches from the varieties. Other physicochemical parameters of the starches such as pH,

ash, fibre and moisture contents were however not compromised. Enzyme treatment also enhanced starch solubility, swelling power, water binding capacities. The technology also rendered the starches lighter in colour. However, extent of interaction of the crude enzymes with mashes (and resulting starches) from the various cassava varieties varied. Thus, the technology impacted the physicochemical parameters (especially regarding swelling power, solubility and amylose content) of starches from some varieties better than those from other varieties. This could influence choice of variety for application of enzyme technology and also greatly impact the utility of the starches extruded. Varietal differences in some physicochemical parameters, with respect to application of enzyme technology, were therefore noted.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Enhancing the retention of β-carotene and vitamin C in dried mango using alternative blanching processes

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The effects of microwave (MW) dry blanching of mango in comparison with conventional water blanching and blanching in closed plastic bags prior to hot air drying (70°C) were evaluated on the retention of vitamin C and β-carotene and on the activity of polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) enzymes. Blanching conditions for MW and water blanching were 2 min at 90°C high temperature and short time (HTST) or for 10 min at 70°C low temperature and low time (LTLT). PPO was completely inactivated by the blanching treatments, while low AAO activity remained. High retentions (~100%) of vitamin C were found in dried mango after blanching treatments HTST with MW and blanching in closed plastic bags, while lower retention was observed after LTLT with MW (81.8 ± 4.5%), and conventional water blanching 86.7 ± 2.6% (HTST) and 78.6 ± 2.5% (LTLT). Blanching resulted in partial oxidation of L-AA into dehydroascorbic acid (DHAA). Lower retention of all-*trans*-β-carotene was obtained in MW and HTST water blanched dried mango (82 to 90%) compared with LTLT water blanched dried mango (~100%). In all dried blanched mango samples the levels of 13-*cis*-β-carotene isomer increased. A slight darkening of colour was observed only in conventional blanched mango samples.

Key words: Microwave heating, mango, β-carotene, vitamin C, ascorbic acid oxidase.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most nutritious tropical fruits, widely consumed in fresh and processed forms. It is seasonal, with relatively short postharvest shelf life due to its perishability (Zhao et al. 2014). Mango can be considered to be a good source of bioactive compounds such as vitamin C, provitamin A carotenoids and phenolic compounds (Ribeiro et al., 2007; Shieber et al., 2000).

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Processing of seasonal fruits and vegetables is desired to prolong their shelf-life and control the activity of enzymes such as polyphenol oxidase (PPO) and ascorbic acid oxidase (AAO) naturally present in fruits and vegetables and responsible for causing quality modifications in texture, flavour, colour and nutritive value (Leong and Oey, 2012; Yamaguchi et al., 2003). The appearance and organoleptic properties of fruits and vegetables are affected by enzymatic browning as the result of polyphenol oxidase (PPO) catalyzed oxidation of mono and diphenols to o-quinones, which are highly reactive compounds subject to further reactions, enzymatically catalyzed or not, leading to the formation of brown pigments (McEvily et al., 1992). Ascorbic acid oxidase (AAO) catalyzes the oxidation of vitamin C in the L-AA form to dehydroascorbic acid (DHAA), which is irreversibly hydrolyzed to 2,3-diketogulonic acid that has no vitamin C activity (Lima et al., 2010). Vieira et al. (2000) reported the degradation of L-AA to DHAA and subsequent degradation during conventional blanching of cupuaçu nectar in the temperature range of 60 to 99°C.

Blanching is the most common method used to stabilize foods via the destruction of microorganisms and inactivation of spoilage enzymes; however, it may lead to a loss of nutritional quality (Puligundla et al., 2013; Ramesh et al., 2002), due to the destruction of vegetable cell membranes during blanching. This causes leaching of water-soluble compounds such as vitamin C during conventional water blanching as well as thermal degradation (Ponne et al., 1994).

Novel heating techniques are gaining more interest in optimizing the quality of processed foods. These involve the use of dry conservation techniques such as microwave and infrared radiation (Vishwanathan et al., 2013; Jeevitha et al., 2013; Lin and Brewer, 2005). Microwave have been applied in a broad range of food processing including blanching, showing a high nutrient retention capacity and minimal loss of heat-labile nutrients such as B and C vitamins, dietary antioxidant phenols and carotenoids (Jeevitha et al., 2015, 2013; Puligundla et al., 2013; Ruiz-Ojeda and Peñas, 2013; Ramesh et al., 2002; Muftugil, 1986). It has advantages compared with conventional heat blanching such as in-depth heating (volumetric) in the absence of a temperature gradient and avoidance of the leaching of water-soluble components (Lin and Brewer, 2005). However, MW lack uniformity in heating and have a limited penetration range (Ramesh et al., 2002; Ramaswamyi and Pillet-Will, 1992).

Microwave (MW) blanching was shown to inactivate oxidative enzymes in papaya, strawberry and kiwi purees with minor losses of carotenoids were detected (De Ancos et al., 1999). Ruiz-Ojeda and Peñas (2013) reported about 50% higher retention of vitamin C in microwaved blanched green bean pods in comparison with conventional hot water treatment. Similar results were reported by Muftugil (1986) and Brewer and Begum (2003) for microwaved green beans and by Ramesh et al. (2002) for spinach, carrot and bell peppers. However, studies on the effect of MW blanching with subsequent conventional drying on the retention of bioactive compounds in fruits and vegetables including mango are lacking.

Thus, the objective of this work was to evaluate the effect of MW dry blanching prior to hot air drying on the retention of vitamin C and carotenoids in dried mango in comparison with conventional water blanching at high temperature and short time (HTST) or at low temperature and long time (LTLT). The remaining activity of PPO and AAO enzymes after blanching was also evaluated.

MATERIALS AND METHODS

The experimental work was performed at the SP Technical Research Institute of Sweden, and at the Division of Food and Nutrition Science, Chalmers University of Technology, in Sweden.

Fruit preparation

Fresh, medium ripe mango (*Mangifera indica* L. cv. 'Osteen'), purchased in a local market and stored at 8 to 10°C, was sliced in a dimmed room into 2 cm diameter and 0.5 cm thick pieces. The average moisture content and water activity of the samples was 0.84 ± 0.02 g/g mango and 0.983 ± 0.002, respectively.

Blanching processes and drying processes

Preliminary tests were performed with 200 g of mango slices to obtain the experimental set-up, given the target combination of time-temperature: 2 min at 90°C (High Temperature Short Time - HTST) or for 10 min at 70°C (Low Temperature Long Time - LTLT) and similar temperature histories for water bath (conventional) or in closed plastic bags and microwave blanching. The temperature of three mango cylinders central point was recorded every second during the heating processes using optic fiber (microwave) or thin-wire copper-constantan thermocouples (water) previously calibrated and connected to a data log. Water blanching was carried out by immersion of a plastic strainer or closed air evacuated plastic bags containing 200 g of sliced mango into thermostatic water bath Julabo Shake Temp SW23, with a 10,000 ml volume of water at constant temperature (70 or 90°C) for a corresponding blanching time (10 or 2 min, respectively).

Microwave blanching was performed using a domestic MW oven (Panasonic NE–C1453, 2450 MHz), with turntable plate. Preliminary studies were carried out to identify the MW conditions (power and time) required for the treatments. Thus, HTST at 90°C required 1350 W/120 s, while LTLT at 70°C demanded a set of power and time sequence of 1350 W/60 s + 420 W/540 s. After blanching, the mango was cooled in iced water for 5 min to lower the residual enzymatic activity, drained and weighed before hot air drying at 70°C using an air circulation oven (Elektro Helios Garomat) with an air velocity of 1 m/s up to a water activity of approximately 0.6. The time required was determined in screening tests and validated by determination of water content and water activity of mango after drying. Samples of fresh, blanched and dried mangoes were collected, filled with nitrogen and stored at -80°C

until determinations of vitamin C, carotenoids and enzymatic activity were carried out. The blanching experiments were performed in duplicate.

Chemicals and standards

All chemicals for extraction and reagents were of analytical or high performance liquid chromatography (HPLC) grade obtained from Sigma–Aldrich (Stockholm, Sweden) or Fischer Scientific GTF (Göteborg, Sweden). The water was generated by Millipore Milli-Q plus an ultra-pure water system (Millipore, Solna, Sweden). All-*trans*-β-carotene standard (synthetic, crystalline, Type II, product C-4582), ACS grade L-Ascorbic acid (≥ 99%), ethylenediaminetetraacetate disodium salt (EDTA) and Tris(2-carboxyethyl) phosphine (TCEP) was obtained from Sigma–Aldrich (Stockholm, Sweden).

Enzyme activity measurements

Enzymatic activities of fresh and blanched mango were determined and the average of three measurements of the duplicates was recorded for both polyphenol oxidase (PPO) and for ascorbic acid oxidase (AAO) enzymes.

Polyphenol oxidase activity

The procedure to measure the PPO enzyme activity in the mango samples has earlier been reported in detail (Guiamba and Svanberg, 2016) and was based on the method described by Palou et al. (1999) and Ndiaye et al. (2009). After extraction of the PPO enzyme using a McIlvaine citric-phosphate buffer (pH 6.5) and addition of a 4-methylcatecol solution, the colour reaction was recorded by measuring the absorbance at 420 nm using a Cary 50Bio UV-visible spectrophotometer.

Ascorbic acid oxidase activity

The activity of AAO in the mango samples was measured according to the procedure earlier described in detail by Guiamba and Svanberg (2016) and based on the method according to Oberbacher and Vines (1963) as modified by Munyaka et al. (2010). The AAO enzyme was extracted from the mango samples using a phosphate buffer (0.1 M, pH 5.6, 0.5 mM EDTA) and the AAO activity was determined by measuring the decrease in substrate concentration (0.5 mM L-AA) using a Cary 50Bio UV-visible spectrophotometer at 265 nm.

Determination of vitamin C (L-AA and total ascorbic acid)

Vitamin C and total ascorbic acid was determined using an HPLC method that was equipped with an electrochemical detector and the procedure was described in detail by Guiamba and Svanberg (2016) with minor modifications. Approximately 1.5 g of fresh or blanched mango and 0.5 g of dried mango was homogenized in an extraction buffer (20 mM NaH_2PO_4, pH 2.1, 1 mM EDTA) and aliquots of the supernatant were diluted both in McIlvaine citric-phosphate buffer (pH 4.5) alone and McIlvaine citric-phosphate buffer containing 0.312 mM TCEP (Tris[2-carboxyethyl]phosphine hydrochloride). TCEP was used to reduce DHAA into L-AA, thereby enabling the determination of both L-ascorbic acid and total ascorbic acid. Results of L-AA and total vitamin C were expressed as milligrams per 100 g dry weight (DW). The percentage retention of vitamin C was calculated as the ratio of vitamin C in the treated sample to the fresh sample × 100.

Determination of β-carotene (*trans/cis* isomers)

Carotenoids analysis was performed using the method described by Bengtsson et al. (2008) with minor modifications as described by Guiamba and Svanberg (2016). Approximately 0.2 g of finely ground flour of freeze dried fresh or processed mango (in duplicate) was added to a test tube and extracted using hexane. After extraction, the carotenoids were analysed by reversed phase HPLC equipped with a C30 polymeric column and a UV–visible photodiode array detector operating at 450 nm. The concentration of each carotenoid was expressed as micrograms per g dry matter, given as the mean of four extractions. The percentage retention of each carotenoid was calculated as the ratio of carotenoid in processed samples to fresh sample × 100.

Analytical determinations

All analytical determinations were performed in triplicate. Water activity was measured using an AquaLab Series 3 – Decagon. The moisture content was measured by drying samples in a vacuum oven at 80°C at 900 mmHg until a constant weight was reached. Colour measurements were carried out using a Digieye verivide (Serie DE00367, Colour Edge CG211, UK) and were based on the CIELAB parameters of L^* (lightness), a^* (redness) and b^* (yellowness). The total colour change (ΔE^*) was the analysed parameter calculated using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where $\Delta x^* = x^* - x_0^*$(white) and x^* representing L^*, a^* and b^*. The results are represented in the normalized form as a ratio ΔE^*dried (or blanched)/ΔE^*fresh.

Statistical analysis

All the tests were performed in triplicate of duplicate samples and the results are presented as mean ± standard deviation. Differences between variables were tested for significance by one-way analysis of variance (ANOVA) and Tukey´s HSD post hoc multiple range test. Differences were considered to be significant at $P < 0.05$ (or at a level of $\alpha = 0.05$).

RESULTS AND DISCUSSION

Temperature profile

Mango cylinders were blanched under similar temperature-time conditions either by microwave (MW) heating or by immersion in a water bath in closed plastic bags or directly in a plastic strainer (conventional) for 10 min at 70°C (Low Temperature Long Time - LTLT) or 2 min at 90°C (High Temperature Short Time - HTST). Samples will be referred as MW70, W70P, W70 and MW90, W90P, W90, respectively. Although blanching in

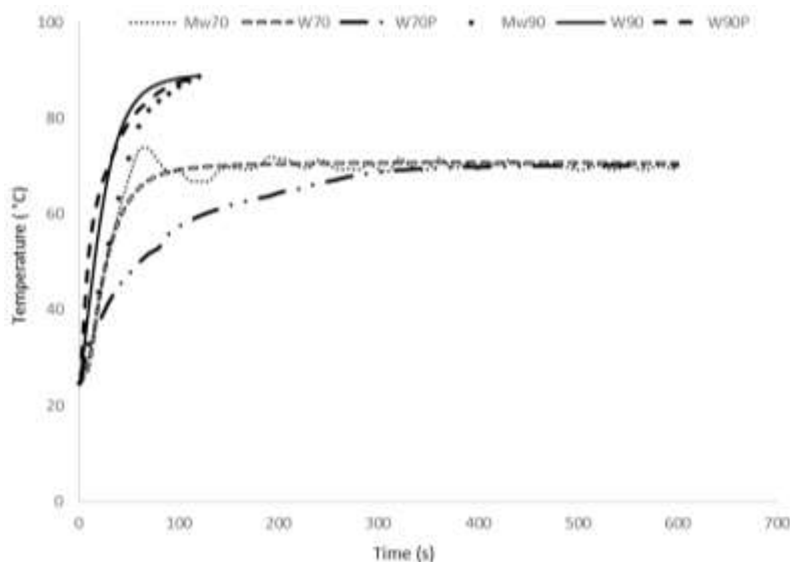

Figure 1. Temperature profiles during LTLT and HTST water and microwave dry blanching.

plastic bags is not a current practice in the industry, it was implemented in the present work to evaluate its possible effect on the retention of nutrients. However, the possibility of its application requires further studies and the identification of suitable material that will not affect the quality of the product. Figure 1 shows the centre temperature profile of mango cylinders obtained during the blanching processes. The increase of mango temperature during water blanching at LTLT was smoother compared to MW blanching due to the relatively low thermal conductivity of mango. A slower temperature increase was observed in mango water blanched in closed plastic bags due to agglomeration of mango cylinders within the plastic bag. On the other hand, the faster temperature increases at the initial stage during MW LTLT heating resulted in a temperature peak at ~75°C, slightly overshooting the set temperature for some few seconds. However, the temperature attained at the end of all blanching processes was 89 and 70°C for HTST and LTLT, respectively.

Enzyme inactivation

The remaining enzymatic activity of PPO and AAO as affected by the blanching processes was evaluated. The initial PPO activity in the fresh mango (n=8) was 68.0 ± 24.4 units, and it was observed that a complete inactivation of polyphenol oxidase (PPO) enzyme was achieved with all blanching processes (data not shown). On the other hand, some residual enzymatic activity of AAO was detected in mango samples after the treatments (Table 1). Minor AAO activity (~6%) remained in the conventional blanched samples (W70) and (W90). Higher activity (12%) was found in microwave blanched samples at high temperature (MW90), 18% at low temperature (MW70) and about 16% in water blanched samples in plastic bags at both temperatures (W70P) and (W90P). The initial AAO activity in the fresh mango was 1.7 ± 0.2 units. Blanching mango of the variety "Tommy Atkins" using conventional water and infrared dry blanching under similar experimental conditions as in the present work resulted in complete inactivation of PPO, and some residual activity of AAO (Guiamba et al., 2015). The results of these studies indicate that AAO in mango seems to be more resistant than PPO. In a comparative study of several blanching methods, Jeevitha et al. (2015) reported that water and steam blanching required less time than microwave, and in turn less than the infrared blanching to inactivate PPO and POD in green bell pepper. This was attributed to a rapid increase in slice temperature during convective heating with water and steam, which led to faster inactivation of the enzymes.

Weight change during blanching and drying time

The results of weight changes that occurred during the blanching treatments are shown in Table 1. It was observed that there was weight loss in all the treatments except for the blanched samples in closed plastic bags since the plastic was a barrier that limited mass change between internal mango samples with the external environment. The weight loss during microwave blanching at low and high temperatures (MW70, MW90)

Table 1. Residual AAO activity (%), blanching weight change (%) and drying time (h).

Parameter	Untreated	MW70	W70	W70P	MW90	W90	W90P
Residual AAO activity	NA	18.2±2.5[a]	6.6±1.8[b]	14.3±4.5[a]	11.9±3.4[a]	6.2±2.0[b]	17.2±5.0[a]
Blanching weight change	NA	-28.0±2.0[a]	-15.5±1.5[b]	-	-15.6±3.6[b]	-7.1±3.2[c]	-
Drying time	1.40±0.00[b]	1.14±0.06[a]	1.35±0.07[b]	1.25±0.07[a,b]	1.10±0.03[a]	1.25±0.00[a]	1.18±0.04[a]

NA: Not applicable. Samples showing the same letter in the same row are not significantly different (p>0.05).

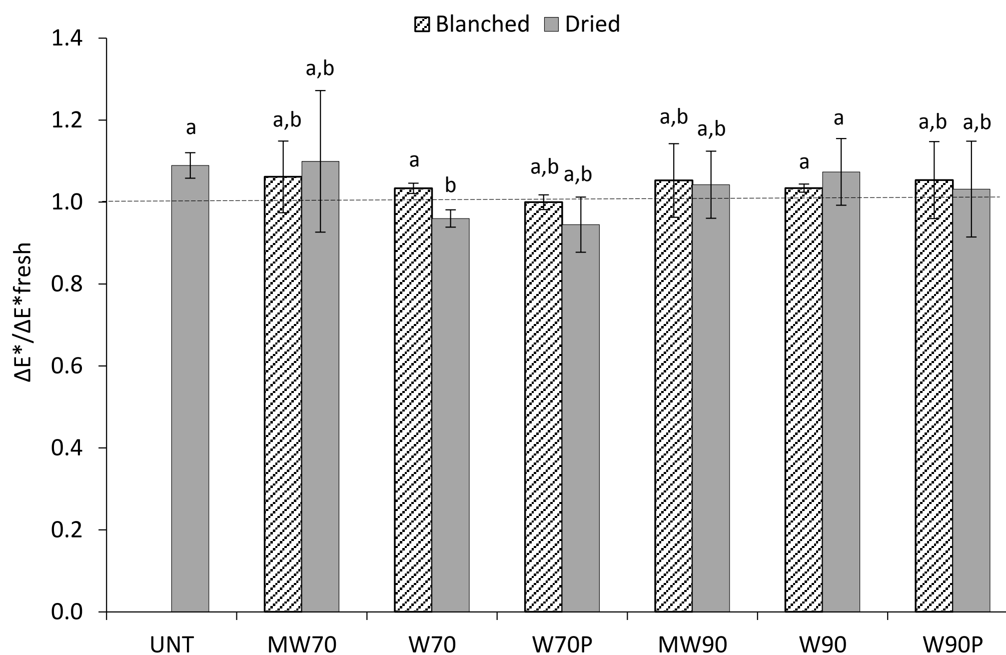

Figure 2. Colour change in dried mango samples relative to the fresh mango presented as ΔE*/ΔE*fresh. Samples showing the same letter are not significantly different (p>0.05). Samples with values >1 are darker while samples with values <1 are lighter.

was significantly higher than with similar water blanching conditions (W70, W90). This was mainly due to the drying action of MW heat, which caused moisture vaporization (Ramesh et al., 2002; Muftugil, 1986). The loss of tissue samples and soluble solids by leaching was thought to be the probable cause of the weight reduction in water blanching. The longer blanching times at 70°C also resulted in higher weight loss when each blanching method was compared separately.

Hot air drying at 70°C was performed for both untreated fresh and blanched mango cylinders in order to reduce the water activity to a level that ensures microbiological stability of the fruit (~0.6). The time required for each process is presented in Table 1. It was observed that a comparatively higher drying time was needed to dry untreated mango samples (1.40 ± 0.00 h) and LTLT water blanched samples (W70 and W70P). MW heated samples showed the lowest drying time. This was due to the relatively higher weight loss during microwave blanching.

Colour

Colour is usually the most influential factor in evaluation of food by consumers, and often the off-colour products are non-acceptable in spite of good taste and flavour (Piližota and Šubarić, 1998). The effect of water and MW thermal treatments, and also combined with hot air drying, as well as drying untreated samples in the colour variation is presented in Figure 2. The total colour difference parameter, ΔE^*, was selected to represent the

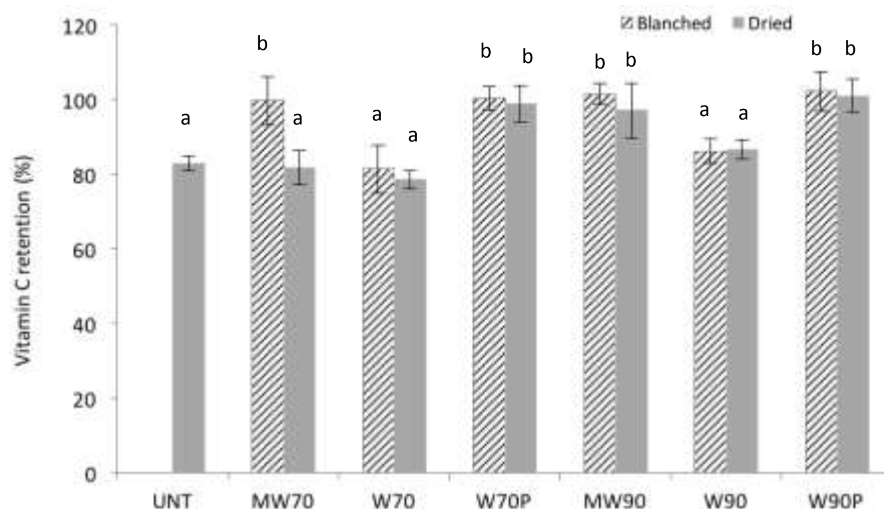

Figure 3. Vitamin C retention in blanched and in dried mango with and without blanching (UNT), as percentage of the amount in fresh mango. Samples showing the same letter are not significantly different (p>0.05).

colour variation of mango, and the results were normalized with the colour of a fresh sample ($\Delta E^*/\Delta E^*$fresh) to compensate for variations in colour between mango from different batches. In comparison with the dried fresh mango, the blanched and dried blanched colour samples was, in general, not affected ($\Delta E^*/\Delta E^*$fresh=1). This could be due to inactivation of PPO during the blanching processes, the enzyme that is responsible for browning (Ioannou and Ghoul, 2013; Palma-Orozco et al., 2012). However, dried untreated (UNT) and conventionally blanched samples (W70, W90) showed some increase in ΔE^* ratio values, as an indication of a slight darkening of these samples, while dried W70 samples resulted in a lower ΔE^* ratio, indicating a lighter colour than in fresh ones. These changes could be related to the thermal treatments, which induce several reactions such as pigment degradation, especially carotenoids, Maillard reactions, and oxidation of ascorbic acid (Barreiro et al., 1997). The Maillard reaction and ascorbic acid oxidation has been shown to produce a yellow brown colour in mango dried at high temperature (70°C) (Chong et al., 2013).

Vitamin C

The total amount of vitamin C (L-AA + DHAA) in fresh mango samples of cv. 'Osteen' was 82.2 ± 27.3 mg/100 g DW. Figure 3 shows the retention of vitamin C in mango after blanching and drying. MW blanching at either high or low temperatures (MW70, MW90) and water blanching in closed plastic bags (W70P, W90P) had no effect on the vitamin C content. The retention after conventional water blanching was however significantly lower, 86.2% (W90) and 81.5% (W70). On the other hand, no further degradation of vitamin C was caused by the drying process, except for MW70 and untreated samples where the retention of 81.8 and 82.9%, respectively was achieved. The degradation of vitamin C in the mango during the drying stage could be associated with a thermal breakdown of the matrix structure (Ponne et al., 1994), which may have caused oxidation of vitamin C in the presence of oxygen.

Compared with conventional treatment, microwave blanching resulted in a better retention of vitamin C, which may be explained by less leaching losses during processing and internal heat penetration by microwaves (Lin and Brewer, 2005; Ramesh et al., 2002). Similar findings of high retention of vitamin C in microwave blanching over conventional treatment have been reported by several authors for green beans (Ruiz-Ojeda and Peñas, 2013; Muftugil, 1986), spinach, bell peppers and carrot (Jeevitha et al., 2015, 2013; Ramesh et al., 2002). The comparable results obtained in this work for MW and water blanching in closed plastic bags may be associated with the limited leaching of water-soluble nutrients in both processes. The analysis of AAO residual activity after blanching versus vitamin C retention in the final dried products did not show a direct relation between these variables.

The total vitamin C is the sum of L-AA and DHAA, both having vitamin C activity (Santos and Silva, 2008). The distribution of vitamin C as L-AA and DHAA in the fresh and dried mango is shown in Figure 4. The retention of vitamin C was mainly as L-AA and the levels were significantly higher in the fresh and dried untreated

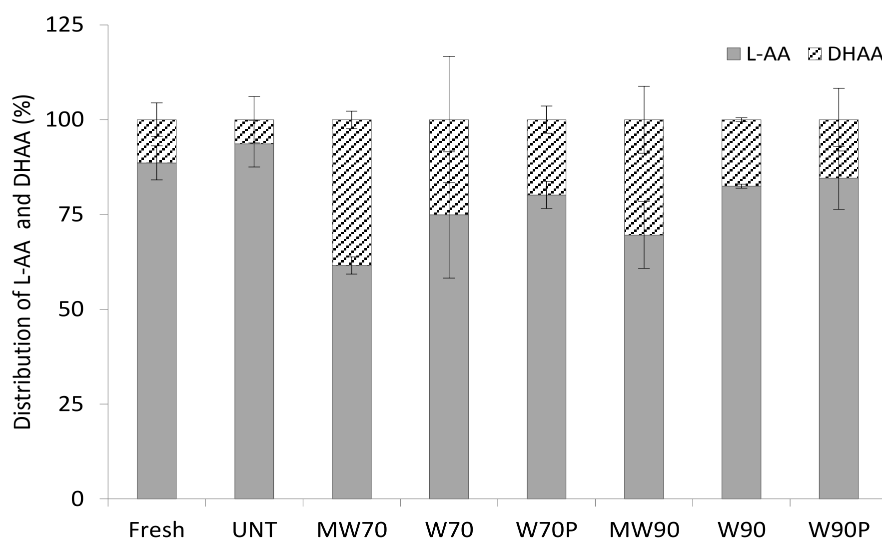

Figure 4. Vitamin C distribution as L-AA and DHAA in fresh and dried mango with or without blanching (UNT).

samples, 89 and 93%, respectively. Higher levels of DHAA were found in the blanched samples. Obviously, the blanching treatments activate the AAO enzyme, resulting in a conversion of L-AA to DHAA. However, there was a trend toward higher levels of DHAA in the LTLT treated samples compared with the HTST treated samples. A possible explanation could be that the lower blanching temperature for the longer time resulted in a slower inactivation of the AAO enzyme and thus a higher oxidation of LAA to DHAA in these samples. Thus, the high level of DHAA in MW70 mango may be associated to the effect of both long blanching time and comparatively higher remaining AAO residual activity (Table 1). Transformation of L-AA into DHAA is influenced by factors such as temperature, light, pH, concentration and metal ions and is facilitated by a naturally occurring enzyme, ascorbic acid oxidase (AAO) in the presence of oxygen (Brewer and Begum, 2003; Davey et al., 2000; Brinkman et al., 1942).

β-carotene

The average amount of total β-carotene in fresh samples, as the sum of *all-trans*-β-carotene and 13-*cis*-β-carotene isomers, was found to be 1759.9 ± 589.6 µg/100 g DW, containing 95.2% (1675.9 µg/100 g DW) of all-*trans*-β-carotene and 4.8% (84.0 µg/100 g DW) of 13-*cis*-β-carotene. These values were in the same range as reported for mango by other authors (Vásquez-Caicedo et al., 2005; Ndawula et al., 2004).

The retention of all-*trans*-β-carotene in the dried mango samples, calculated in relation to the original amount in fresh mango (DW), is shown in Figure 5. The retention of all-*trans*-β-carotene in LTLT water blanched dried samples (W70 and W70P) was not affected by the pre-treatment. However, in dried mango that was water blanched (W90 and W90P), the retention was higher (~90%) compared with the microwave blanched mango (MW70 and MW90) where the retention decreased to values of about 82%. Severe degradation of all-*trans*-β-carotene was noted in dried untreated (UNT) mango, with a retention of around 57%. The explanation might be the PPO activity causing an indirect co-oxidation of the all-*trans*-β-carotene when exposed to high temperature in the presence of oxygen (Xianquan et al., 2005; Dorantes-Alvarez and Chiralt, 2000).

Contrary to the results on vitamin C, the retention of all-*trans*-β-carotene in mango blanched using plastic bags did not differ from the mango conventionally water blanched. This may be associated to the fact of carotenoids being water-insoluble pigments but lipid soluble (Mao et al., 2009). In addition, carotenoids are highly unsaturated and prone to isomerization and oxidation during processing and storage due to the effects of chemical, mechanical and thermal stresses (Qian et al., 2012; Mao et al., 2009; Boon et al., 2009). Thermal processing of carotenoid containing foods with cooking oil has been shown to significantly reduce the retention of all-*trans*-β-carotene and to increase the level of 13-*cis*-β-carotene (Bengtsson et al., 2010). In mango, the β-carotene is embedded in lipid droplets (Brackmann et al., 2011; Bartley and Scolnik, 1995), which then may explain the lower retention in mango subjected to a high blanching temperature (90°C).

Microwave blanching of other plant foods than mango

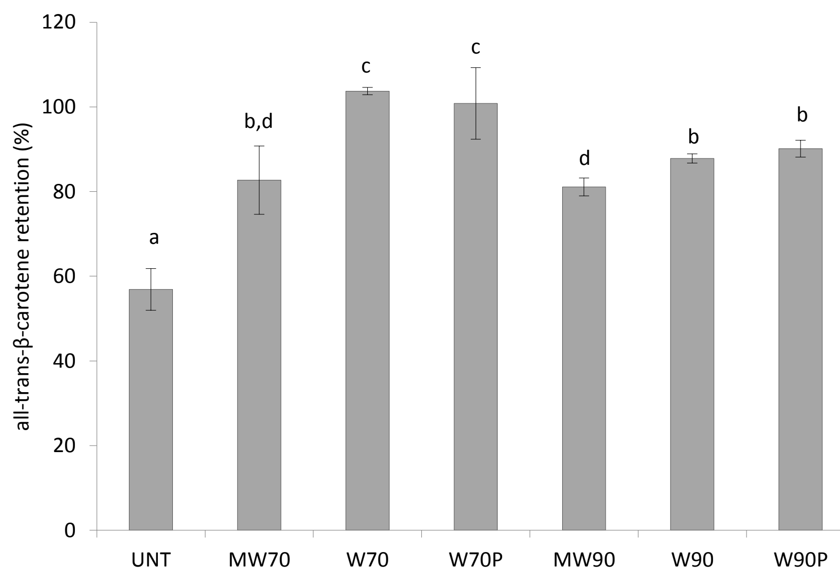

Figure 5. All-*trans*-β-carotene retention in dried mango with or without blanching (UNT). Samples showing the same letter are not significantly different (p>0.05).

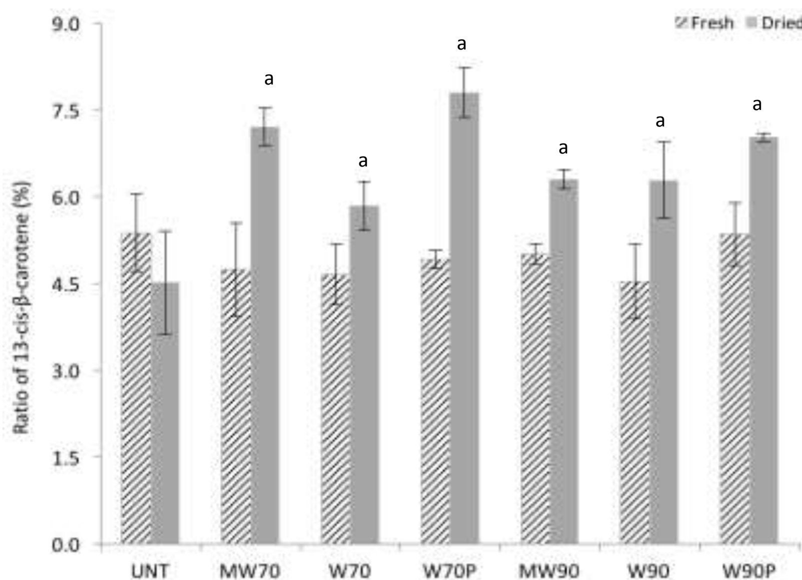

Figure 6. The percentage ratio of 13-*cis*-β-carotene to all-*trans*-β-carotene in relation to fresh and dried mango with or without blanching (UNT). Dried blanched samples with the letter a are significantly different (p<0.05) from the corresponding fresh mango.

has been reported in the literature. According to De Ancos et al. (1999), microwave heating induced a loss of total carotenoid content in papaya purée as high as 57%. Opposite results were reported by Jeevitha et al. (2015, 2013), i.e., a retention of more than 100% of β-carotene in green and red bell peppers after microwave blanching, which was attributed to an enhanced extractability of nutrient from blanched samples.

The presence of *cis*-β-carotene isomers in fresh mango has been reported by several authors (Chen et al., 2007; Pott et al., 2003; Godoy and Rodriguez-Amaya, 1994). The ratio of 13-*cis*-β-carotene (%) to all-*trans*-β-carotene in the fresh and dried mango samples as affected by the treatments is presented in Figure 6. The ratio of 13-*cis*-β-

carotene remained unchanged in untreated (UNT) mango after drying. However, a significant increase in the ratio of this isomer was observed in all dried blanched samples. These results suggest that the thermal treatment (blanching) affected the stability of all-*trans*-β-carotene, leading to isomerization. Reports in the literature show that heat treatment promotes isomerization of the carotenoids in foods, from *trans* to *cis* isomeric forms, and that the degree of isomerization is directly correlated with the intensity and duration of heat processing (Lemmens et al., 2013; Achir et al., 2011; Vásquez-Caicedo et al., 2007; Rodriguez-Amaya et al., 2006; Chen and Huang, 1998) and with the presence of oil in the process (Bengtsson et al., 2010).

Conclusions

Selection of suitable technology and conditions for blanching can improve the nutritional value of dried mango and inactivate relevant enzymes. Complete inactivation of PPO in mango was achieved with heat treatments at 90°C for 2 min (HTST) and at 70°C for 10 min (LTLT), while AAO showed a low remaining activity for the conditions tested. A higher retention of vitamin C (water-soluble, heat and oxygen sensitive) in dried mango was achieved using MW dry blanching or water blanching in plastic bags in comparison to conventionally water blanching, due to the limited leaching of water-soluble compounds in these processes. The distribution of vitamin C as L-AA and DHAA in fresh and dried mango was mainly as L-AA, but with relatively higher amounts of DHAA in blanched samples, thus showing an influence of blanching on the oxidation of L-AA to DHAA.

LTLT or HTST blanching before drying is an effective way to improve the retention of carotenoids (water insoluble, prone to isomerization and oxidation) in dried mango. The retention of all-*trans*-β-carotene was higher in LTLT water blanched dried samples compared with HTST, while the corresponding MW blanched dried mango had lower retention. Moreover, blanching in plastic bags did not differ from water conventional blanching. MW blanching showed no significant difference between LTLT and HTST treatments. In all pre–blanched mango, the levels of 13-*cis*-β-carotene isomer were higher than in the fresh samples.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Evaluation of thigh muscles of 3 breeds of cattle (White Fulani, Sokoto Gudali and Red Bororo)

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If different breeds or muscles of animal have same composition is a food for thought. Do muscles from animals eaten as food, have different chemical composition? This study evaluates composition of thigh muscles from three breeds of cattle and the different muscles in the thigh. Nine 1-year male cattle, comprising of 3 Sokoto Gudali (SG), 3 White Fulani (WF) and 3 Red Bororo (RB) breeds were reared semi-intensively, fed with concentrate and allowed to grazed for 10 weeks. Each breed thigh and their muscles {semi-membranous (SM), semi-tendinosis (ST), Gracilis (GR), Sartorius (S), Vastus Lateralis (VL), Tensor Fascia tatae (TL) and Biceps Femoris (BF)} were evaluated for proximate, minerals and palatability status, in a completely randomized design. Results of breeds, show that SG had highest significant ($P<0.05$) protein, magnesium, iron and phosphorus contents and lowest ether extract content. Out of all the muscles, SM had best proximate composition while VL had best mineral composition than other muscles significantly evaluated. Physico-chemical analysis showed that cooking loss was lowest ($P<0.05$) for SG (32.68%) than WF (39.61%) and RB (35.15%). For muscles, ST, BF, SM and GR had highest significant ($P<0.05$) water holding capacity values of 62.72, 55.46, 57.65 and 52.34%, respectively than 39.76 (VL), 42.19 (S) and 42.90% (TL). With regards to palatability scores for breeds, panelists scored SG highest ($P<0.05$) than WF and RB. For muscle, SM was scored best ($P<0.05$) with highest significant values for flavor, tenderness, texture and overall acceptability. SG appeared best in all the breeds evaluated, while SM did well in proximate, VL and ST in minerals, SM in physico-chemical and palatability status.

Key words: Breeds of cattle, Red Bororo, Sokoto Gudali, thigh muscles, White Fulani.

INTRODUCTION

Consumers purchase beef or meat and produce them into desirable products or are cooked into recipes or soup without having full knowledge on the breeds, type of muscles or nutritional value they might get from the product bought. Recent findings have shown that muscles from same cattle have different nutritive values and organoleptic qualities. Sanuelo et al. (2004) noted that animal breeds can influence the nutritive qualities of

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meat in some ways, which could affect the muscles structure and meat physiology.

Researchers have shown that some important aspect vary significantly between muscles and within the same muscle from animal irrespective of their diets (Biesalski, 2005). Muscles in animal body consist of 3 types, skeletal, smooth and cardiac muscles tissues which vary with function and location in the body. Skeletal muscles are muscles of the muscular system, having over 600 in an animal body, the amount used to produce human food represents 35 - 60% of their body weight and vary widely in shape, size, anatomical locations, physiological functions, action and nutritional composition. Listrat et al. (2016) reported that muscle mass of livestock used to produce human food represents 35 – 60% of their body weight. The Meat We Eat.Com (2017) observed that the most abundant chemical in meat is water, followed by protein then fat. Carbohydrates, minerals and vitamins occur in much smaller amounts but nevertheless are very important metabolically and nutritionally.

There are different muscles in skeletal system like the thigh, the shoulder, the loins, etc. Major muscles found in animal thigh include semi–membranous, semi–tendinosis, Gracilis, Sartorius, Vastus Lateralis, tensor fascia latae, and biceps femoris muscles according to Tomaszewska-Gras and Piotr (2012). The differences in this thigh muscles will dictate differences in sensory and nutritive value of the meat produced and one thigh muscle may be different from another thigh muscle of the same animal type. Therefore, the aim of this study is to assess the thigh muscles from three breeds of cattle and the differences in the muscles of thigh of an animal.

MATERIALS AND METHODS

Experimental animals

Nine male live animals of a year old, comprising of 3 White Fulani, 3 Sokoto Gudali and 3 Red Bororo were purchased from Osun State University Teaching and Research Farm. Thereafter, the cattle were quarantined, dewormed, given anti-stress and antibiotics. The animals were raised under a semi-intensive housing system for ten weeks and tagged for easy identification (White Fulani– WF, Sokoto Gudali– SG, Red Bororo– RB). Immediately after exsanguination, skinning methods were applied (removal of the skin) in order to identify the thigh muscles for the experiment.

Identification of muscles in the thigh

Each muscle obtained was identified in the meat laboratory as semi–membranous (SM), semi–tendinosis (ST), Gracilis (Gr), Sartorius (S), Vastus lateralis (VL), tensor fascia latae (TL) and biceps femoris (BF).

Proximate and mineral composition

Proximate composition

Proximate (protein, ether extract, ash and moisture contents) samples and minerals (magnesium, calcium and phosphorus) samples were analyzed chemically according to the Official Analytical Chemist (AOAC, 18TH EDITION, 2005).

Physico-chemical evaluations

Water holding capacity

The WHC of meat samples was determined by the press method as slightly modified by Suzuki et al. (1991). Approximately 1 g of meat sample was placed between two (9 cm Whatman No1) filter papers (Model C, Caver Inc, Wabash, USA). The meat sample was then pressed between two 10.2 x 10.2 cm^2 Plexi glasses at about 35.2 kg/cm^3 absolute pressure for 1 min using a vice. The meat samples were removed and oven dried at 80°C for 24 h to determine the moisture content. The amount of water released from the meat samples was measured indirectly by measuring the area of filter paper wetted relative to the area of pressed meat samples. This procedure was repeated for all the seven muscles in each cattle. Therefore, the WHC was calculated thus:

$$\mathrm{WHC} = 100 - \{(\mathrm{Aw} - \mathrm{Am} \times 9.47)\}$$

$$Wm - Mo$$

Where, Aw = Area of water released from meat samples (cm^2); Am = area of meat samples (cm^2); Wm = weight of meat samples (g); Mo = moisture content of meat samples (%); 9.47 = a constant factor.

Cold shortening

Meat samples of known weight and length were stored in refrigerator for 24 h at 4°C (Fakolade et al., 2016). After refrigeration, the final length were taken and calculated thus:

$$\text{Cold } shortening\ \% = \frac{(Initial\ length\ of meat - length\ of frozen\ meat)}{Initial\ length\ of meat} \times 100$$

Thermal shortening

Meat samples of known length and known weight were taken and broiled in an oven at 175°C for 20 min (Fakolade et al., 2016). Afterwards, the final length was taken and calculated thus:

$$Thermal\ shortening\ (\%) = \frac{(Initial\ length\ of\ meat - length\ of\ frozen\ meat)}{Initial\ length\ of\ meat} \times 100$$

Cooking Loss

Meat samples of known length and weight were taken and broiled at 175°C for 20 min (Fakolade et al., 2016). Afterwards, the final weight was taken and calculated thus:

$$Cooking\ loss\ (\%) = \frac{(Initial\ weight\ of\ meat - weight\ of broiled\ meat)}{Initial\ weight\ of\ meat} \times 100$$

Thaw rigor

Meat samples of known weight and length were stored in refrigerator for 24 h at 4°C. After refrigeration, the final weight were

Table 1. Proximate and mineral composition of thigh muscles from Sokoto Gudali, White Fulani and Red Bororo breeds of cattle.

Variable	SG	WF	RB	SEM
Protein	20.6[a]	19.2[b]	20.0[a]	1.40
Ash	1.09	1.03	1.11	0.14
Ether extract	5.42[b]	7.02[a]	5.93[b]	0.10
Moisture	72.5	72.7	73.0	0.21
Magnesium	87.4[a]	84.00[b]	86.16[a]	0.21
Iron	27.4[a]	23.54[b]	27.54[a]	0.16
Phosphorus	280[a]	271[b]	277[b]	0.40

[ab]: Means of different alphabet along the column are significantly different (P<0.05). SG- Sokoto Gudali, WF– White Fulani, RB– Red Bororo.

Table 2. Proximate and mineral composition of different muscles in the thigh of cattle.

Variable	GR	BF	S	VL	TL	ST	SM	SEM
Protein	21.0[ab]	18.6[c]	19.5[b]	19.0[b]	19.1[b]	20.1[ab]	22.1[a]	0.21
Ash	1.05	1.07	1.12	1.10	1.15	0.98	1.08	0.02
Ether E	7.57[a]	5.77[b]	6.26[b]	6.07[b]	5.79[b]	5.72[b]	5.68[b]	0.16
Moisture	73.6	72.0	71.5	73.7	72.8	72.9	72.5	0.32
Phosphorus	278[b]	273[c]	272[c]	282[a]	270[d]	282[a]	273.8[c]	0.61
Magnesium	84.7[b]	85.0[b]	83.8[b]	90.2[a]	85.8[b]	88.4[ab]	82.8[b]	0.21
Iron	26.1[b]	26.1[b]	23.7[c]	30.5[a]	24.8[d]	28.7[a]	23.4[c]	0.25

[ab]: Means of different alphabet along the column are significantly different (P<0.05). GR– Gracilis, BF– biceps femoris, S– sartorius, VL– vastus lateralis, TL– tensor fascia latae, ST– semi tendinosus, SM– semi membranosus.

taken and calculated thus:

$$Thaw\ Rigor\ (\%) = \frac{Initial\ weight\ of\ meat - weight\ of\ frozen\ meat)}{Initial\ weight\ of\ meat} \times 100$$

Palatability status

A total number of forty trained panelists aged 27 to 45 years were selected based on their past performance in consuming meat, and were randomly allocated to the samples. Each panelist was presented the blind coded samples and asked to score each sample for flavour, tenderness, juiciness, colour and overall acceptability (Fakolade et al., 2016).

Statistical analysis

The experimental design adopted was complete randomized design (CRD). All data were subjected to analysis of variance (ANOVA) and means were separated with Tukey HSD using the same analytical software.

RESULTS AND DISCUSSION

SG had the highest significant protein value of 20.6% than RB (20.0%) and WF (19.2%). It also had the highest mineral composition value (P<0.05) than the others for magnesium, iron and phosphorus. The values obtained for proximate composition agrees with the report of Adeniyi et al. (2011) that beef protein ranges from 18 to 22%, moisture 68 to 75% and fat 2 to 15%. The values obtained for ash in this study were below 1.19% as observed by Dixon et al. (2015) for beef. SG had the highest mineral composition measured followed by RB and then WF. SG had been observed by most consumers to have better quality value for processing into products, which could be well explained by Table 1.

SM appeared best significantly in values for proximate analysis, while VL and ST took the lead in mineral composition (P<0.05) values. The values obtained were in the ranges of 21.17 to 23.21%, as reported by USDA (2012) for SM muscle of beef. SM and ST are chucks of muscle that have laboratory values, easy to manage and process (Table 2).

SG had the highest WHC but lowest values for other parameters measured. This indicates that SG meat is of good quality, having retained more of it nutritional quality. Drummond et al. (2005) observed that higher cold shortening value in meat could make the meat tougher and Kauffman (1992) reported that higher thaw rigor reduces the juiciness, texture and nutrient in meat (Tables 3 to 6).

SM was proven to be the best muscle evaluated from

Table 3. Physico-chemical status of thigh muscles from Sokoto Gudali, White Fulani and Red Bororo breeds of cattle.

Variable	SG	WF	RB	SEM
Cooking Loss	32.6^{b}	39.6^{a}	35.1^{b}	2.22
Thermal Shortening	03.5^{c}	06.0^{b}	09.1^{a}	2.17
Thaw Rigor	01.2^{b}	5.68^{a}	5.62^{a}	1.88
Water Holding Capacity	54.8^{a}	44.7^{b}	43.1^{b}	3.94
Cold Shortening	14.8^{b}	35.0^{a}	20.2^{b}	1.88

^{ab}: Means of different alphabet along the column are significantly different (P<0.05). SG– Sokoto Gudali, WF– White Fulani, RB– Red Bororo.

Table 4. Physico-chemical status of different muscles in the thigh of cattle.

Variables	BF	ST	SM	GR	VL	S	TL	SEM
Cooking loss	33.6^{c}	35.1^{bc}	32.5^{c}	30.3^{c}	36.9^{bc}	43.7^{a}	32.1^{c}	3.40
Thermal Shortening	19.0^{a}	02.1^{d}	09.5^{c}	12.0^{b}	17.0^{a}	06.9^{c}	11.9^{b}	3.60
Thaw Rigor	06.4^{c}	09.2^{b}	11.3^{b}	17.7^{a}	09.1^{b}	02.5^{d}	06.0^{c}	3.60
Water Holding Capacity	55.4^{b}	62.7^{a}	57.6^{ab}	51.3^{b}	39.7^{d}	42.1^{c}	42.9^{c}	3.37
Cold Shortening	23.6^{b}	30.6^{a}	23.8^{b}	21.1^{b}	18.5^{c}	33.5^{a}	23.1^{b}	2.88

^{ab}; means of different alphabet along the column are significantly different (P<0.05). BF– Biceps femoris, ST– semi tendinosus, SM– semi membranosus, GR– gracilis, VL– vastus lateralis, S– sartorius, TL– tensor fascia latae.

Table 5. Palatability scores of thigh muscles from Sokoto Gudali, White Fulani and Red Bororo breeds of cattle.

Variable	SG	WF	RB	SEM
Colour	5.55^{a}	4.73^{b}	4.99^{a}	0.19
Flavour	5.36	5.31	5.54	0.17
Tenderness	5.16^{a}	4.80^{b}	5.28^{a}	0.17
Texture	5.64^{a}	5.38^{a}	4.98^{b}	0.20
Juiceness	5.57^{a}	5.58^{a}	4.76^{b}	0.18
Acceptability	7.20^{a}	4.98^{c}	5.72^{b}	0.18

^{ab}: Means of different alphabet along the column are significantly different (P<0.05). SG– Sokoto Gudali, WF– White Fulani, RB– Red Bororo.

Table 6. Palatability score of different muscles in the thigh of cattle.

Variable	BF	ST	SM	GR	VL	S	TL	SEM
Colour	4.76^{b}	5.56^{a}	4.58^{b}	4.94^{b}	5.04^{ab}	4.72^{b}	4.84^{b}	0.30
Flavour	5.24^{a}	5.30^{a}	5.76^{a}	5.26^{a}	5.81^{a}	5.65^{a}	4.77^{b}	0.27
Tenderness	5.76^{a}	4.82^{b}	5.39^{a}	4.44^{b}	4.49^{b}	5.69^{a}	4.94^{b}	0.26
Texture	5.04^{b}	5.21^{b}	6.15^{a}	5.29^{b}	4.67^{c}	6.19^{a}	4.90^{c}	0.28
Juiciness	6.19^{a}	5.37^{b}	6.41^{a}	5.07^{b}	4.82^{c}	5.43^{b}	5.03^{b}	0.32
Acceptability	5.47^{b}	5.68^{b}	6.64^{a}	5.67^{b}	5.86^{b}	5.83^{b}	5.29^{b}	0.27

^{ab}: Means of different alphabet along the column are significantly different (P<0.05). BF- Biceps femoris, ST- semi tendinosus, SM- semi membranosus, GR- gracilis, VL – vastus lateralis, S- sartorius, TL- tensor fascia latae.

the seven muscles. It had the highest WHC, and the lowest values for other parameter measured. This shows how quality such muscles could be. Hoffman et al. (2012) reported that cooking loss of meat cold reduce the quality

of meat, and obtained 34.22 to 45.64% of cooking loss for beef animal. Other parameters like thermal, cold shortening and thaw rigor do reduce the quality of any meat if it is of higher value.

The panelists scored SG better than WF and RB ($P<0.05$). This may be due to the fact that SG had the highest significant value in proximate and minerals composition, with lower physico-chemical parameters with good WHC which could influence other organoleptic characteristics like texture, juiciness and tenderness of the meat.

SM had the best taste organoleptically, as scored by the panelists. This could also be due to the fact that, it appeared best for proximate analysis done, having good physico-chemical qualities. Fakolade et al. (2011) indicated that meat with higher flavor, texture and juiciness will motivate the panelists to score higher overall acceptability.

Conclusion

SG proved to be the best quality breed of cattle from those evaluated, while SM muscle gave the best nutritional qualities than the other muscles analyzed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Biochemical changes in superchilled storage of salmon (*Salmo salar*) fillets

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Biochemical changes of Atlantic salmon (*Salmo salar*) fillets during superchilled storage were evaluated. Due to the significant differences in ice crystal sizes observed, the biochemical changes were evaluated both at the top and centre parts of the superchilled samples. No significant differences were found in biochemical changes between top and centre parts of the superchilled samples. The amount of cell tissue fluid (CTF) increased significantly from day 1 to 3 in the top and centre parts of the superchilled samples. The amount of CTF was stable between day 3 and 14 but increased significantly from day 21 to 28. A significant increase in the activity of β-N-acetyl-glucosaminidase in the CTF at the top was observed between day 3 and 7, while at the centre at day 7 of storage. There was also a significant increase in β-N-acetyl-glucosaminidase both at the centre and the surface of the superchilled samples between day 21 and 28 of storage whereby the activity in these samples was on the same level as in the frozen samples. In the superchilled samples, the cathepsin B activity in CTF and homogenates were stable for the first one week of storage. There was significant increase in activity of cathepsin B in cell tissue fluid and homogenates between day 21 and 28 for both chilled and superchilled samples. These findings provides valuable information on the quality of food products for the food industry in relation to ice crystallisation/recrystallisation during superchilled storage.

Key words: Partial freezing, biochemical changes, superchilled storage, salmon fish (*Salmo salar*).

INTRODUCTION

Partial freezing of food is an effective way of preservation and it has been shown to double shelf-life of foods (Duun and Rustad, 2008; Duun et al., 2008). Superchilling and superchilled storage of food may, however, lead to quality changes and protein denaturation, especially of the myofibrillar proteins. These changes may result in altered functional properties, such as changes in texture, reduced water holding capacity and juiciness. These properties are related to food quality and are important for maintaining high quality.

Quality is an arbitrary term and one which causes confusion among consumers, processors and researchers. Product quality is a very complex concept (Gao, 2007) which included: Nutritional, microbiological,

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biochemical and physiochemical attributes. Quality not only relates to the freshness of food, how old it is and the appearance, it also related to the service received by the consumers and the consumer's expectations and specifications (Gao, 2007). Microbial growth, colour, taste, texture, water holding capacity and juiciness, off-flavour, biochemical properties and oxidation are important factors both for the safety and the quality of food products. The deterioration in the food quality during superchilled storage is due to undesirable processes taking place in lipids and proteins (Duun and Rustad, 2008; Jiang and Lee, 1985; Ocano-Higuera et al., 2009). The degree of deterioration is influenced by many factors such as freshness and treatment before partial freezing, state of rigor, partial freezing rate, ultimate partial freezing temperature, storage temperature, storage period, fluctuation of storage temperature, and thawing methods (Benjakul and Visessanguan, 2010; Blond and Meste, 2004; Chevalier et al., 2001; Duun and Rustad, 2008; Duun et al., 2008; Hagiwara et al., 2002; Jiang and Lee, 1985; Kiani and Sun, 2011; Martino et al., 1998; Mittal and Griffiths, 2005; Petzold and Aguilera, 2009).

There is growing demand for fresh and high quality foods worldwide. In recent years, consumers are increasingly concerned with their health, and are demanding foods that are beneficial to their health and help prevent diseases. This leads to an increased need for technologies to preserve the freshness of foods. Superchilled storage is an efficient way of preserving the quality of food (Kaale et al., 2013b). With good handling and safety practices both before and after partial freezing, superchilled foods can retain high quality. To retain good quality, the superchilled foods should be kept at stable superchilled storage temperatures (Banerjee and Maheswarappa, 2017). At these temperatures, growth of micro-organisms is very low and deteriorative reactions take place at very slow rates (Duun and Rustad, 2008).

Nevertheless, studies by Kaale et al. (2013b) and Kaale et al. (2013c) reported that a high superchilling rate results in a high rate of heat removal, leading to the formation of a large number of small nuclei and thus a large number of small ice crystals that grow both within and outside cells. As a result, the cells maintain their integrity which in turn minimises drip loss; maintain water holding capacity and textural changes during thawing (Smith, 2011). However, this advantage was reduced during superchilled storage by the rapid growth of ice crystal sizes in the salmon fillets (Kaale et al., 2013b). Moreover, there was a large difference between the ice crystal sizes at the surface and centre of the superchilled salmon fillets. The increase in ice crystal sizes during superchilled storage may impart mechanical damage by physically rupturing cell walls, which may result in an increase in drip loss, a reduction of the water-holding capacity and textural changes (Smith, 2011).

Therefore, there is a need to study the damage which may take place during superchilled storage of salmon fillets. When muscle foods are partially frozen, cell and organelle membranes are destroyed leading to leakage of lysosomal enzymes into the cell (Nilsson and Ekstrand, 1993). The degree of membrane destruction can be measured as an increase in amount of cell tissue fluid (CTF). Increase in lysosomal enzyme activity in the CTF is also a measure of membrane destruction. Increased amount of CTF is closely related to loss of water holding capacity and increase with reduced ability of the tissue to hold water (Duun and Rustad, 2008; Johansen, 2013). Hence, the objective of this study was to assess cell tissue fluid and enzyme activity changes both at the surface and centre of the salmon fillets in order to clarify the effect of the ice crystal development during superchilled storage.

MATERIALS AND METHODS

Salmon fillets (*Salmon salar*) stored in ice (0.9 to 1 kg), were delivered by Lerøy Midnor (Hitra, Norway). The fillets were vacuum-packed and stored at 4°C for 24 h before the superchilling process to ensure a constant temperature in all samples. Superchilling was performed in an Impingement Advantec Lab Freezer (JBT Food-Tech, Rusthållsgatan 21, SE-251 09, Helsingborg, Sweden) at NTNU Energy's laboratory in Trondheim, Norway. The samples were superchilled (partially frozen) at -30°C and 227 $W/m^2.K$ (at 2.5 kPa pressure differences of the fan at the impingement freezer) for 2.1 min to achieve an ice content of 20% (Kaale et al., 2013c).

Temperature trend during storage

The temperature, as one of the critical parameters during superchilled storage, was strictly controlled during this study. The storage box was designed (92 × 73 × 54.5 cm) with a heating element inside to ensure adequate temperature regulation. Three Pt100 temperature sensors were inserted in the storage box: One was used to measure the air temperature, and the other two were used to measure the surface and centre temperatures of the superchilled salmon fillets. The set-point temperature was -1.7°C. The box was placed inside the storage room, which was at a temperature of approximately -5°C (temperature outside the storage box).

Cell tissue fluid (CTF)

Approximately 20 g of coarsely cut muscle was weighed out in a centrifuge tube and centrifuged at 4°C for 30 min at ~28 000 *x g* in SS-34 rotor (Sorvall centrifuge, The Netherlands) (Nilsson and Ekstrand, 1994). The supernatant was pipetted off, and this is the CTF. Quantification of the CTF was made by weighing directly into Eppendorf tubes. Samples were kept at -80°C until analysis. Two parallels were made from each sample.

Protein extraction (homogenate preparation)

Extraction of water soluble protein was performed as described by Nilsson and Ekstrand (1994) at +4°C, and samples were kept on ice before, under and after homogenization. Each sample was extracted once. For protein fractionation, approximately 5 g of

muscle was cut from the loin side and weighed out in a centrifuge tube (50 mL), and 15 mL of 50 mM phosphate buffer, pH 7.0 was added before homogenization (Ultra Turrax). The homogenate was left in a cold room for 30 min, occasionally stirred or shaken before centrifugation at 4°C for 30 min at ~14 700 *x g* in a SS-34 rotor (Sorvall centrifuge). The supernatant was decanted into a volumetric flask, through a funnel with glass wool. The glass wool was rinsed with buffer, and the volume was made up to 25 ml. Aliquots of the extracts were frozen at -80°C until analyses.

Enzymatic activity

Activity of the lysosomal enzymes cathepsin B and N-acetylaminidase were determined in CTF and extract of water soluble proteins. The cathepsin B activity was determined with a synthetic fluorogenic substrate, N_{α}-carbobenoxy-L-arginyl-L-7-amido-4-methylcoumarin (Barrett and Kirschke, 1981). Activity of β-N-acetylglycosaminidase (NAG) was determined using ρ-nitrophenyl-N-acetyl-β-D-glucose amide as a substrate. The activity was determined as described by Milanesi and Bird (1972).

Statistical analysis

The observations of the CTF at the two locations with respect to storage days were analysed by one- and two-way analyses of variance using Minitab 16 software (Minitab, Inc., USA). A general linear model, (post- hoc test) under Tukey's simultaneous test was applied whenever the ANOVA results were significant ($p < 0.05$).

RESULTS AND DISCUSSION

Ice crystal evolution during the superchilling process and the superchilled storage

Figure 1 showed the micrographs of superchilled and without superchilled salmon tissues (Control). The study by Kaale et al. (2013a) reported that the ice crystal size in zero-time (superchilling process) was significantly smaller (23 ± 3 μm) compared to that during storage of superchilled samples (92 μm), which is 4 folds larger than ice crystals at zero time. This is because the superchilling process was performed at a very low temperature (-30°C) and the samples were stored at -1.7 ± 0.3°C.

In addition, thermal gradients which developed during the superchilling process in the product near the surface were reported to contribute to the increase of ice crystal size during superchilled storage. When temperature equalisation was achieved within the samples, the growth of the intracellular ice crystals at the surface layer was not significant ($p < 0.05$) (Kaale et al., 2013a). Prior to temperature equalisation, ice crystal growth progresses from the surface to the centre of the superchilled food products (Figure 1). No ice crystals were formed at the centre during the superchilling process (zero time). The size of ice crystal that are formed at the centre was significantly larger than that at the surface (318 ± 4 μm_ after only 1 day of storage, which is 3-folds larger than ice crystals at surface. The large size is due to slow superchilling rate, and these crystals may damage the integrity of the superchilled product. Due to the significant difference in ice crystal sizes (surfaces vs centres) within the superchilled salmon samples, cell tissue fluid and enzyme activity were analysed separately at the surfaces and centres of the fillets during superchilled storage.

Cell tissue fluid (CTF)

Figures 2 and 3 showed the amount of CTF at the centre and surface of the superchilled samples. The amount of CTF increased during storage. No significant differences were found in CTF between the top and centre parts of the superchilled samples. Kaale et al. (2013a) reported that the temperature equalization had taken place at day 1 and there was no significant growth of ice crystal size after this time. In this study, there was a significant increase in amount of CTF from day 1 to 3 at the top and the centre parts of the superchilled samples. The amount of CTF was stable between day 3 and 14 but increased significantly from day 21 to 28.

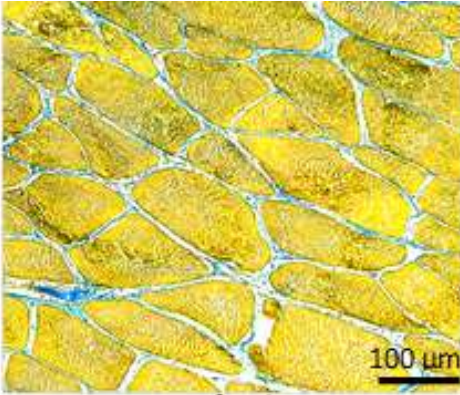
The changes in CTF showed a slight damage of cell membranes between day 1 and 3 where temperature equalization takes place and between day 21 and 28 (Figure 2). These results are according to that reported in previous study (Kaale et al., 2014). The type of samples and storage procedures in this study are the same as that used in Kaale et al. (2014).

Enzyme activity

Leakage of lysosomal enzymes such as different cathepsins, β-N-acetyl-glucosaminidase and α-glucosidase have been used as an indicator of membrane damage due to freezing (Nilsson and Ekstrand, 1993; 1994, Bahuaud et al., 2008). The significant increase in the activity of β-N-acetyl-glucosaminidase in the CTF at the top was observed between day 3 and 7, while at the centre on day 7. In contrast, the β-N-acetyl-glucosaminidase in the CTF increased significantly between day 21 and 28 at the centre and the surface of the superchilled samples and the activity in these samples (day 21 and 28) was on the same level as in the frozen samples. No significant differences were found in the β-N-acetyl-glucosaminidase in the CTF between top and centre parts of the superchilled samples.

The activity of β-N-acetyl-glucosaminidase in extracts of water soluble proteins (Figure 4) from the chilled samples increases between day 7 and 14 of storage while in the superchilled samples there is a decrease between day 7 and 14 for the top samples and at the centre was observed at day 14 of storage, followed by an increase to the same level as in the samples from day one. This could imply that there are inhibitors that are inactivated during chilled storage while this does not take place till after 14 days of superchilled storage.

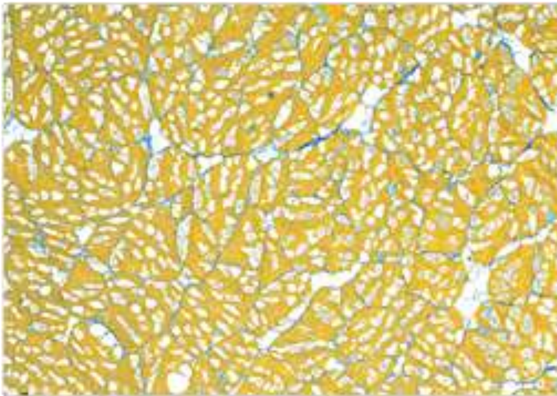
Storage days

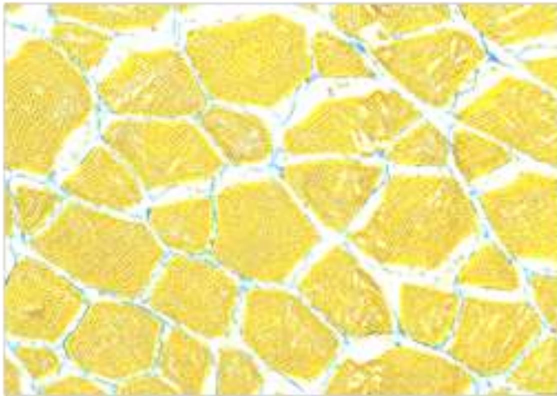


Control

Storage days | Surface | Centre

0





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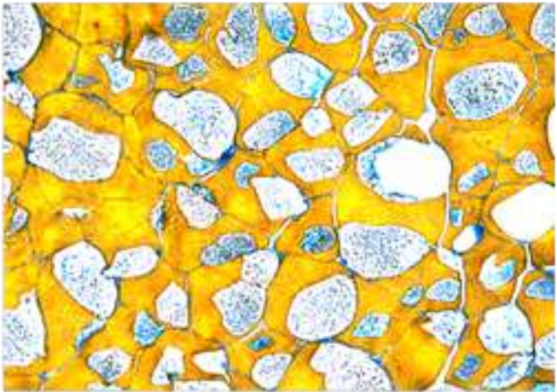

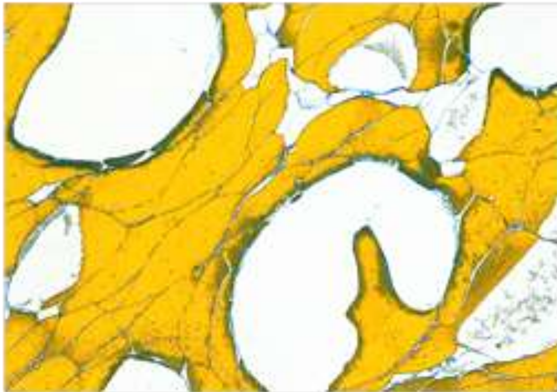

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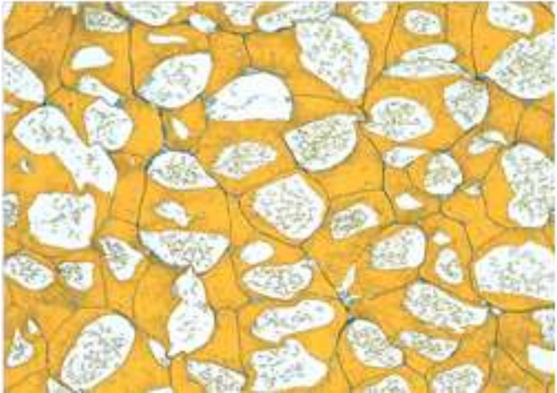

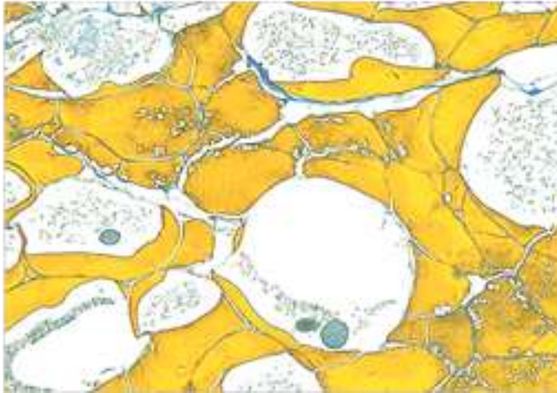

Figure 1. Micrographs of superchilled and without superchilled salmon tissues.

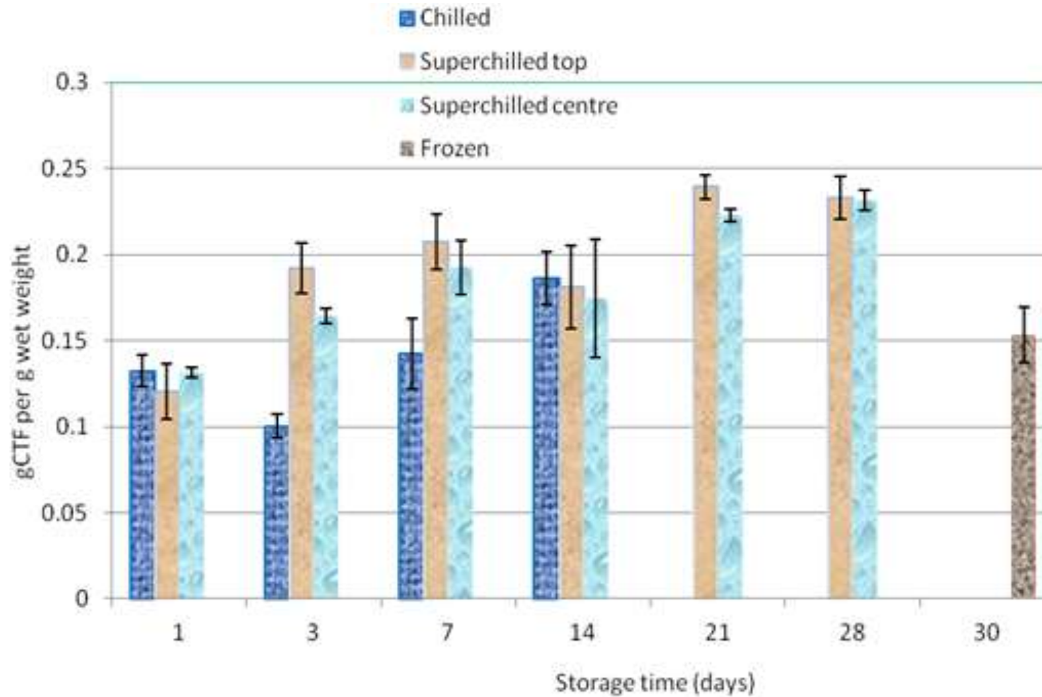

Figure 2. Changes in amount of cell tissue fluid per gram wet weight in salmon under different storage conditions. Superchilled storage at -1.7°C, chilled storage at 4°C and frozen at -20°C. Values are given as mean ± SEM of (g CTF / g wet weight) (n =3).

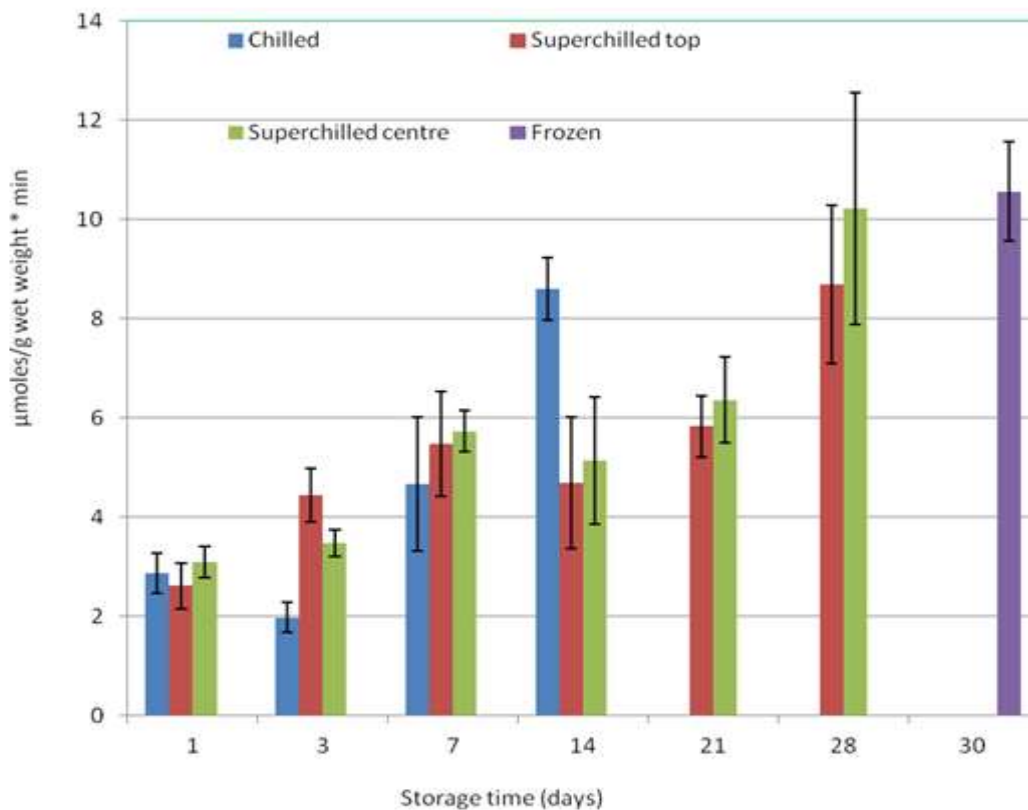

Figure 3. Changes in activity of β-N-acetyl-glucosaminidase in cell tissue fluid from salmon. Superchilled storage at -1.7°C, chilled storage at 4°C and frozen storage at -20°C. Values given as mean ± SEM (µmoles / g wet weight*min) (n = 3).

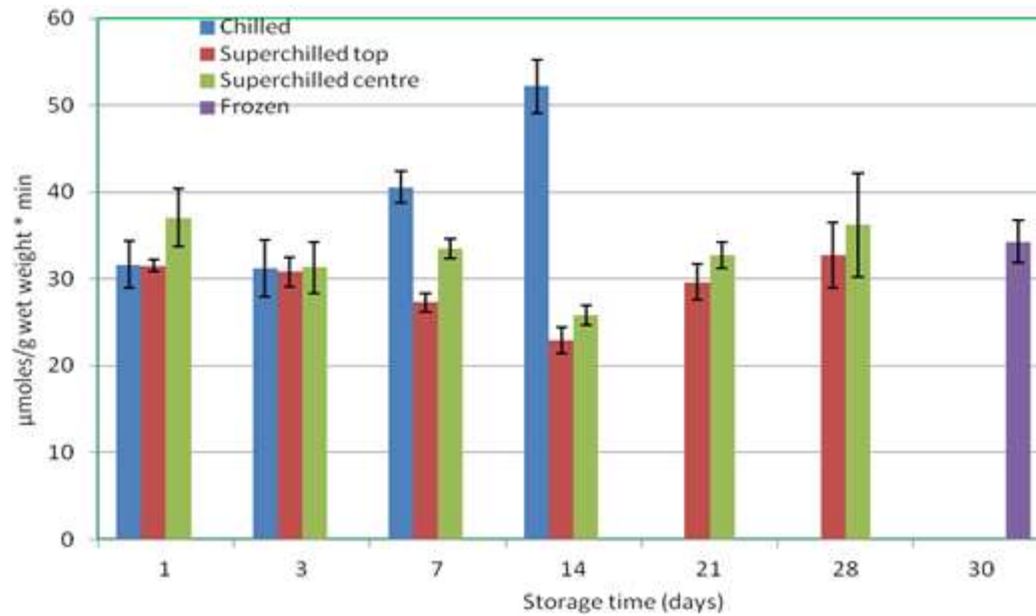

Figure 4. Changes in activity of β-N-acetyl-glucosaminidase in homogenates from salmon. Superchilled storage at -1.7°C and chilled storage at 4°C. Values are given as mean ± SEM of (µmoles/ g wet weight*min) (n =6).

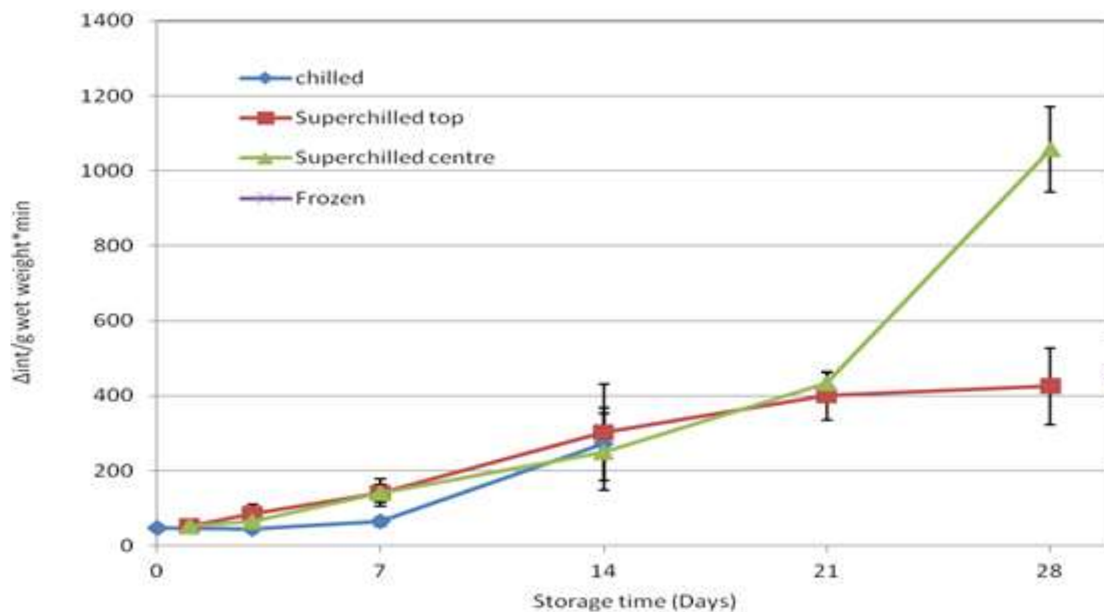

Figure 5. Changes in activity of cathepsin B in cell tissue fluid from salmon at different storage period and conditions. Superchilled storage at -1.7°C, frozen storage at -20°C and chilled storage at 4°C. Values are given as mean ± SEM (n= 6).

Cathepsins are also lysosomal enzymes, and the cathepsin B activity in CTF (Figure 5) was stable for the first one week of storage in all samples. There was a significant increase in activity of cathepsin B in cell tissue fluid between day 21 and 28 for chilled and superchilled samples which might be due to cell denaturation during superchilled storage.

For the homogenates (Figure 6), there is a slight increase in the activity for the chilled samples while the activity in the superchilled samples decrease during the

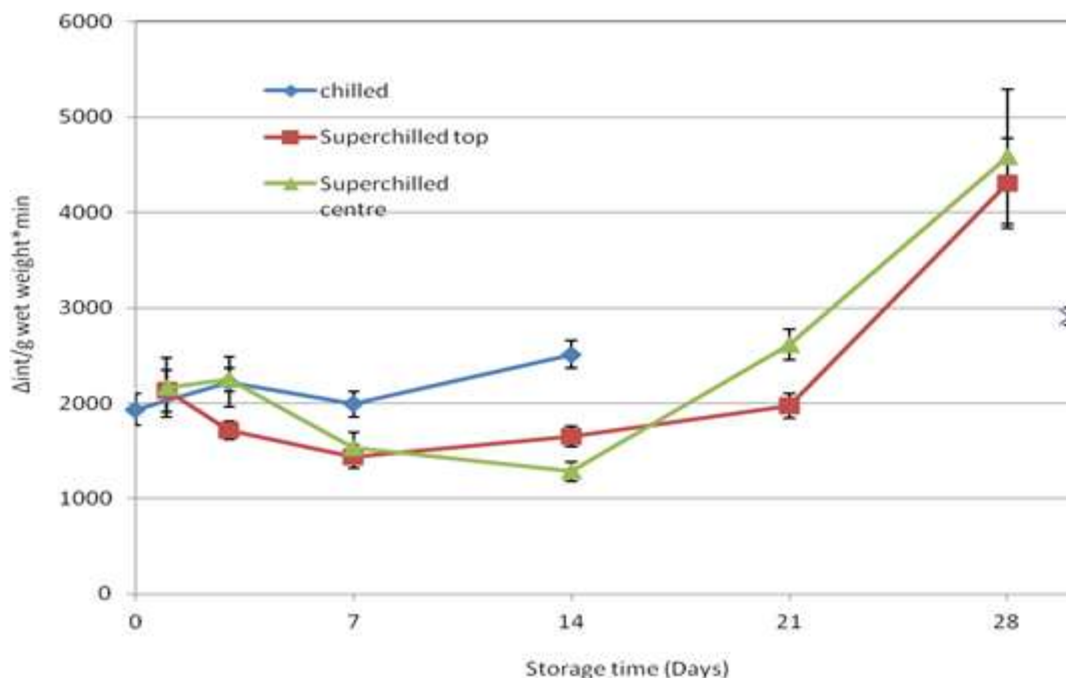

Figure 6. Changes in activity of cathepsin B in homogenates from salmon at different storage period and conditions. Superchilled storage at -1.7°C frozen storage at -20°C and chilled storage at 4°C. Values are given as mean ± SEM (n= 6).

first 1 to 2 weeks and then increase from day 21 to 28.

No significant differences were found in cathepsin B activity in CTF between top and centre parts of the superchilled samples.

Conclusion

No significant differences were found in biochemical changes between top and centre parts of the superchilled samples. The amount of CTF increased significantly from day 1 to 3 in the top and centre parts of the superchilled samples. The amount of CTF was stable between day 3 and 14 but increased significantly from day 21 to 28. A significant increase in the activity of β-N-acetyl-glucosaminidase in the CTF at the top was observed between day 3 and 7, while at the centre at day 7 of storage. There was also a significant increase in β-N-acetyl-glucosaminidase in both the centre and the surface of the superchilled samples between day 21 and 28 of storage whereby the activity in these samples is on the same level as in the frozen samples. In the superchilled samples, the cathepsin activity in CTF and homogenates were stable for the first one week of storage. There was significant increase in activity of cathepsin B in cell tissue fluid and homogenates between day 21 and 28 for both the chilled and superchilled samples. These findings provides valuable information on the quality of food product for the food industry in relation to ice crystallisation/recrystallisation during superchilled storage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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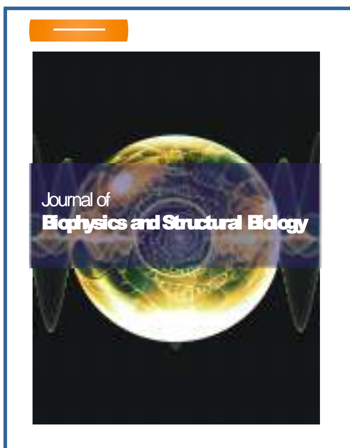

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