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Optimising drying conditions for maximum nutritional quality and bioactivity of *Cucurbita pepo* L var. *fastigata* flesh and seeds

Nulu Nansikombi[1] John H. Muyonga[2*] and Yusuf B. Byaruhanga[2]

1, *Department of Food Science and Technology, School of Food Technology, Nutrition and Bio-engineering, Makerere University, Kampala, Uganda*
2, *Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bio-engineering, Makerere University, Kampala, Uganda*

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***Corresponding author:**
Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bio-engineering, Makerere University, Kampala, Uganda.
Email: hmuyonga@yahoo.com

ABSTRACT

Purpose: Transformation of pumpkin flesh and seeds into dry flours increases their shelf stability and versatility. This study sought to optimise drying conditions for production of flour with high nutritional and nutraceutical value from flesh and seeds of *C. pepo* L var. *fastigata* using Response Surface Methodology, I optimal design. **Research Method**: Pumpkin flesh and seeds were dried following temperature time combinations got using I optimal design. All dry samples were tested for ascorbic acid, total carotenoids, total antioxidant capacity and starch digestibility. Seeds were also tested for *in vitro* protein digestibility, trypsin inhibitor activity, alpha tocopherol, beta tocopherol, and alpha tocotrienol. **Findings**: The predicted optimum drying conditions for production of *C. pepo* L var. *fastigata* flour with maximum nutritional quality and bioactivity were 57°C; 6.9 hours for flesh and 60°C; 3.15 hours for seeds. The most influential model terms were temperature for resistant starch, *in vitro* protein digestibility, trypsin inhibitor activity, total carotenoids, alpha tocopherol, beta tocopherol and alpha tocotrienol; quadratic term of temperature and time for ascorbic acid; and drying time for total antioxidant activity of the flesh and temperature for antioxidant activity for seeds. **Research limitations**: Dryer air flow rate was not included as a variable. **Originality/Value:** Results give, for the first time, an objective basis for choice of the drying conditions *C. pepo* L var. *fastigata* flesh and seeds for maximum nutritional and health benefits.

INTRODUCTION

Pumpkin (*Cucurbita* spp) flesh is rich in carbohydrates and carotenoids while seeds are rich in protein, fat and bioactive compounds such as carotenoids, vitamin E and sitosterol (Kim et al., 2012). Drying of pumpkin flesh increases shelf-life and versatility, enabling use in bakery products, sauces, pasta and instant noodles (Mirhosseini et al., 2015).

Hot air drying is widely used in production of pumpkin powder (Que et al., 2008). Several studies have assessed the effect of drying temperature and methods on properties of dried products. However, there is lack of information on the optimum pumpkin drying temperature-time combinations. Roongruangsri and Bronlund (2016) reported significant effect of drying temperature on the physicochemical attributes of pumpkin powder. Ceclu et al. (2016) found significant differences in rehydration properties of pumpkin slices dried using different methods. Monteiro et al. (2018) reported effect of drying method on drying kinetics, rehydration kinetics and rehydration indices of dehydrated pumpkin slices. The current study sought to determine the effects of hot air drying conditions (including temperature and time) on preserving the nutritional quality and bioactivity in the flesh and seeds of *C. pepo* L var. *fastigata*.

MATERIAL AND METHODS

Mature fruits were cut into 8 pieces using a stainless steel kitchen knife, seeds removed, pieces peeled and sliced to 0.3 cm thick and 2 cm long. Seeds were washed under running tap water for about 5 minutes. Preliminary drying experiments were conducted at 40 to 80°C using an electric dryer (Philip Harris Ltd, Shenstone England) and limits for temperature and time set based on attainment of about 10% moisture content (Ekorong et al., 2015). Samples were picked at intervals of 30 minutes for flesh and 15 minutes for seeds and moisture content determined using the oven method (Bradley, 2003). The selected drying limits were 40-80°C and 3.5-11 hours for flesh and 40-80°C and 1.25-5.25 hours for seeds (Table 1).

Response Surface Methodology I optimal design (Design Expert version 11 Stat-Ease Inc., Minneapolis MN) was used to get 17 independent randomised drying temperature-time combinations (experimental runs) (Table 2) for drying samples. Samples were milled (multi-function mill Model 100, Zhejiang Winki Plastic Industry Co., China) and analysed for different response variables. Numerical optimisation was used to establish the optimum drying temperature-time. Triplicate samples were dried using optimal conditions, milled and resulting flours analysed. The prediction power of the developed models was verified by comparing the theoretical predicted data to the experimental data.

Table 1. The experimental levels of drying conditions used in the I-optimal design

Sample	Factor			Factor range and values		
	Symbol	Name	Units	-1	0	+1
Flesh	A	Temperature	°C	40.00	60	80.00
	B	Time	Hours	3.50	7.5	11.50
Seeds	A	Temperature	°C	40.00	60	80.00
	B	Time	Hours	1.25	3.25	5.25

Table 2. Experimental Conditions to which pumpkin flesh and seeds were subjected

Flesh samples			Seeds samples		
Experimental run	Temperature (°C)	Time (Hours)	Experimental run	Temperature (°C)	Time (Hours)
1	50	9.5	1	50	3.25
2	70	11.5	2	50	4.25
3	70	7.5	3	70	5.25
4	50	11.5	4	70	4.25
5	60	7.5	5	50	4.25
6	50	7.5	6	60	4.25
7	70	5.5	7	60	3.25
8	40	11.5	8	80	1.25
9	70	9.5	9	70	3.25
10	60	9.5	10	70	2.25
11	60	5.5	11	60	5.25
12	60	7.5	12	40	5.25
13	50	9.5	13	50	3.25
14	50	7.5	14	60	3.25
15	60	11.5	15	60	3.25
16	60	7.5	16	50	5.25
17	80	3.5	17	60	2.25

Starch digestibility

Moisture content of the raw samples and flours was determined using the oven method (Bradley, 2003) and resistant starch determined following megazyme resistant starch assay procedure (Megazyme International, 2017). Flour (0.1 g) and raw sample (0.5 g) were each placed in a falcon tube and 4 mL of a solution containing pancreatic α-amylase (10 mg.Ml^{-1}) and 3 enzyme units of amyloglucosidase per milliliter (3 U.mL^{-1}) added. One enzyme unit (U) being the amount of enzyme required to convert one micromole of a substrate under specified conditions of the assay method. The mixture was covered, vortexed, aligned horizontally in a shaking water bath (100 rpm; 37°C), for 16 hours, 4 mL of 99% ethanol added, vortexed and centrifuged at 1,610×g for 10 minutes (225 centrifuge, Fisher Scientific Co. St. Louis, MO). The supernatant was decanted off, 2 mL of 50% ethanol added to the pellet, vortexed, 6 mL of 50% ethanol added, mixed and centrifuged (1500×g; 10 minutes). The supernatant was decanted off, 6 mL of 50% ethanol added, mixed, centrifuged (1500×g; 10 minutes) and decanted. To the pellet, 2 mL of 2 M potassium hydroxide was added, stirred for 20 minutes, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) and 0.1 mL concentrated amyloglucosidase solution (3,300 U.mL^{-1}) added, mixed, incubated in a water bath (50°C; 30 minutes) with intermittent vortexing, and the mixture adjusted to 100 mL using distilled water. An aliquot was centrifuged (1500×g; 10 minutes), 0.1 mL supernatant transferred into duplicate test tubes, 3 mL of glucose oxidase peroxidase (GOPOD) added, incubated (50°C; 20 minutes) and the absorbance read at 510 nm against a reagent blank. Quadruplicate glucose standards (0.1 mg.mL^{-1}) were mixed with 3 mL GOPOD, the absorbance determined, and resistant starch calculated as follows (1).

$$\text{Resistant starch (g/100g, dwb)} = \Delta A \times F \times \frac{100\text{ mL}}{0.1\text{ mL}} \times \frac{1}{1000} \times \frac{100}{W\text{ (g)}} \times \frac{162\text{ (g)}}{180\text{ (g)}} \quad (1)$$

Where:

ΔA: absorbance read against the reagent blank
F: conversion factor from absorbance to micrograms of glucose

$$F = \frac{100(\mu g \text{ of standard glucose sloution})}{\text{Absorbance of standard glucose sloution}}$$

100/0.1: volume correction factor since 0.1mL was taken from 100mL
1/1000: conversion from micrograms to milligrams
W: dry weight (g) of the sample analysed

$$W\ (g) = \text{"as is" weight (g)} \times \left[\frac{100 - \text{moisture content (\%)}}{100}\right]$$

$\frac{100}{W\ (g)}$: factor expressing nonresistant starch as a percentage of sample
$\frac{162\ (g)}{180\ (g)}$: conversion factor from free $D-$ glucose, as determined, to anhydro $-D-$ glucose as occurs in starch

In vitro protein digestibility

The multi enzyme method of Hsu et al. (1977) as modified by Park et al. (2010) was used. Protein content of the raw seeds and of the flours was determined using the AOAC 955.04 micro Kjeldahl method (AOAC, 1995). A test solution containing 6.25 mg protein/mL was prepared and its pH adjusted to 8.0 using 0.1 N sodium hydroxide. An enzyme solution of 1.6 mg trypsin (T0303), 3.1 mg chymotrypsin (C4129) and 1.3 mg peptidase (P4762 (all from SIGMA ALDRICH USA) was prepared, its pH adjusted to 8.0 using 0.1 N sodium hydroxide, 1 mL added to a 10 mL sample, mixed, incubated in a shaking water bath (130 rpm; 37 °C) for 10 minutes, pH determined and protein digestibility calculated as follows (2).

$$\text{Protein digestibility (\%)} = 201.464 - 18.103A \quad (2)$$

A: the final pH

Trypsin inhibitor activity

Trypsin inhibitor activity was determined following the bromocresol purple index "BCPI" method as reported by Szmigielski et al. (2010). Bromocresol purple index (quantity of bromocresol purple adsorbed, expressed as per protein mass unit in dry matter) was determined as follows (3).

$$BCPI = \frac{(A_b - A_s) \times C \times V}{A_b \times M} \times \frac{P}{100} \quad (3)$$

A_b: absorbance of the reagent blank
As: absorbance of the sample
C: concentration of bromocresoe dye (mg/mL)
V: volume of bromocresoe dye solution used (50mL)
M: mass of the sample (g)
P: percent protein content of the sample (dry matter basis)
Trypsin inhibitor activity (TIA mg/g protein DMB) was determined as follows (4).

$$TIA\ (mg/g) = -1.3042\ (BCPI) + 27.049 \quad (4)$$

α-tocopherol, β-tocopherol and α-tocotrienol content

α-tocopherol, β-tocopherol and α-tocotrienol were determined as described by Frick and Doyle (2015). To a 0.4 g sample in a 50 mL centrifuge bottle, 10 mL of methanol were added, mixed, 10 mL of HPLC grade mixture of ethyl acetate and hexane (1:1) added, vortexed for

20 minutes, centrifuged (4,251×g; 10 minutes), the organic (upper) layer transferred into a clean tube and the sediment re-extracted with 10 mL of extracting solvent. The filtrates were combined, reconstituted to 100 mL and a 5 µL aliquot analyzed using Agilent Technologies 6420 Triple Quad LCMSMS system (SG16217007, Singapore). The HPLC conditions were a carbon 18 column (Poroshell 120 EC-C18, 2.7 µm, 3.0x50 mm (B16163), USA), two mobile phases composed of a mixture of 0.1% formic acid and 5 mM ammonium formate (A) and 0.1% formic acid (B) flowing at 0.5 mL.minute^{-1}. The separation was performed at 40°C. The Mass spectrometer conditions were gas temperature of 250 °C flowing at 8 L.minute^{-1}.

Total carotenoids

A sample (2 g) was extracted with 50 mL cold acetone in the dark, distilled water (250 mL) slowly added, the aqueous phase discarded and the procedure repeated four times. The extract was filtered through glass wool containing 15 g anhydrous sodium sulphate into a 50 mL volumetric flask, adjusted to volume using petroleum ether and its absorbance measured at 540 nm against petroleum ether as the blank (Rodriguez-Amaya & Kimura, 2004). The total carotenoid content was calculated as follows (5).

$$\text{Total carotenoid}(\mu g/g) = \frac{\text{Absorbance} \times \text{Total volume of extract} \times 10^4}{2592 \times \text{sample weight (g)}} \quad (5)$$

Where;
2592: absorption coefficient of beta carotene

Ascorbic acid content

Ascorbic acid content was determined using the 2, 6 Dichloroindophenol method (AOAC, 1995). Extracts of dark coloured samples were decolorised prior titration by adding a spatulaful of activated carbon to 10 mL followed by centrifugation (906×g; 10 minutes).

Total antioxidant activity

A 1 g ground sample was extracted thrice using 5 mL of 50% methanol while filtering, the filtrate pooled and volume adjusted to 100 mL with 50% methanol (Muanda et al., 2009). Antioxidant activity of the extract was determined by mixing 50 µL extract with 2.9 mL of freshly prepared 50% methanolic solution of 100 µM DPPH, vortexed, allowed to stand in the dark, at room temperature for 30 minutes and the absorbance of the mixture and control (DPPH solution) measured at 515 nm against 50% methanol as blank (Stratil et al., 2006). Antioxidant activity (percent radical scavenging activity) was calculated as follows (6) (Marwah et al., 2007).

$$\text{Radical scavenging activity (\%)} = \left[1 - \left(\frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}}\right)\right] \times 100 \quad (6)$$

Statistical analysis

To determine the optimum drying conditions, all statistics and construction of 3D graphs were generated using the Design-Expert software (version 11, Stat-Ease Inc., Minneapolis MN). I-optimal design of RSM was used to fit polynomial models for the different response variables. The best model suggested by the program (one with highest order polynomial and p-value <0.05, was obtained from the Fit summary. The goodness of fit of the chosen model was determined using the coefficient of determination (R^2 close to 1) and a non-significant p-value for lack of fit (p >0.05). Analysis of variance at 5% level of significance was done in order to determine the significant model terms (P ≤0.05).

RESULTS AND DISCUSSION

The predicted optimum conditions for drying of *C. pepo* L var. *fastigata* flesh and seeds were 57°C; 6.9 hours and 60°C; 3.15 hours respectively. For both the flesh and seeds, all models (equations 7 to 19) were significant (P<0.05) and all experimental values were within the confidence interval of the respective predicted values (Table 3). Furthermore, all models had a non-significant lack of fit implying that the models were valid.

Starch digestibility

High drying temperature and long drying time resulted in increased resistant starch content of the flesh (Figure 1A) and seeds (Fig. 1B). Based on the coefficient of determination (R^2) values, 94.9% (equation 7) and 97% (equation 8) of sample variation for resistant starch of the flesh and seeds, respectively, was attributed to the independent variables namely temperature (A) and drying time (B).

$$\text{Resistant starch (g/100g sample DMB)}_{\text{Flesh}} = 36.12 + 0.40A + 0.10B - 0.12AB - 0.05A^2 - 0.05B^2 \text{ (model P value} < 0.001, R^2 = 0.949, \text{lack of fit P value } 0.563) \quad (7)$$

$$\text{Resistant starch (g/100g sample DMB)}_{\text{Seeds}} = 1.34 + 0.14A + 0.01B + 0.02AB + 0.02A^2 + 0.03B^2 \text{(model P value} < 0.001, R^2 = 0.970, \text{lack of fit P value } 0.287) \quad (8)$$

For the flesh (Table 4) and seeds (Table 5), temperature significantly and positively affected the resistant starch content. Donlao and Ogawa (2017) reported an increase in resistant starch content of rice as drying temperatures increased from 40 to 90°C and a decrease beyond 115°C. The increase in resistant starch of flesh and seeds could be due to drastic loss of moisture at drying temperatures not high enough to cause melting of the starch granules. This is supported by Vamadevan and Bertoft (2015) who report that heating a starchy food in presence of limited water like during drying, shifts the gelatinisation peak to a higher temperature. This inhibits gelatinisation thus reducing starch digestibility. Oh et al. (2018) also reported a negative correlation between dry heating temperature of 110 to 130°C and the *in vitro* starch digestibility of high amylose rice starch.

Table 3. Optimum predicted versus experimental values at the optimised drying

Response	Flesh			Seeds		
	Predicted values		Experimental Value	Predicted values		Experimental value
	Mean	95% CI for Mean		Mean	95% CI for Mean	
RS (g.100g^{-1} sample)	36.04	36.01-36.08	35.99±0.04	1.33	1.31-1.34	1.30±0.02
PD (%)				66.25	65.69-66.81	65.43±0.82
TIA (mg.g^{-1})				5.68	5.55-5.80	5.41±0.28
TAA (%DPPH scavenged)	35.55	33.74-37.33	35.72±0.97	44.75	44.41-45.09	44.63±0.41
AA (mg.100g^{-1})	43.58	42.01-45.15	41.88±0.46	14.42	14.33-14.50	13.89±0.52
TC (mg.100g^{-1})	11.52	11.07-11.97	10.61±0.55	7.00	6.94-7.06	6.56±0.44
α-tocopherol (mg.100g^{-1})				6.51	6.43-6.59	6.47±0.06
β-tocopherol (mg.100g^{-1})				2.92	2.89-2.94	2.83±0.08
α-tocotrienol (mg.100g^{-1})				3.36	3.34- 3.38	3.30±0.05

Data are means of triplicate determinations ± standard deviation.
(Resistant starch: RS; Protein Digestibility: PD; Trypsin inhibitor activity: TIA; Total antioxidant activity: TAA; Ascorbic acid: AA; Total carotenoids: TC)

Table 4. Significance of the nutrient quality and bioactivity parametric model terms for the dried pumpkin flesh

Factor	Total antioxidant activity			Total carotenoids			Ascorbic acid content			Resistant starch		
	CE	SE	P-value	CE	SE	P-value	CE	SE	P-value	CE	SE	P-value
Intercept	35.22	0.69		11.57	0.18		43.46	0.65		36.12	0.01	
A	2.63	2.22	0.274	1.15	0.42	*0.020	2.82	1.47	0.081	0.40	0.03	*<0.001
B	-6.97	2.08	*0.012	-1.13	0.43	*0.023	-5.26	1.49	*0.004	0.10	0.03	*0.012
AB	-4.84	5.53	0.410	0.29	0.72	0.689	-0.89	2.51	0.728	-0.12	0.05	*0.047
A^2	-7.82	3.99	0.090	-2.53	0.74	*0.006	-12.61	2.57	*<0.001	-0.05	0.06	0.360
B^2	-1.74	4.19	0.691	0.45	0.56	0.440	2.43	1.96	0.241	-0.05	0.04	0.260
A^2B	0.33	7.82	0.967									
AB^2	4.13	6.13	0.522									
A^3	-6.43	7.98	0.446									
B^3	3.95	4.48	0.407									

Model terms with P<0.05 are significant and are assigned an asterisk (*); A: Temperature; B: Time; CE: coefficient estimate and SE: standard error.

Table 5. Significance of the nutrient quality parametric model terms for the dried pumpkin seeds

Factor	Protein digestibility			Trypsin inhibitor activity			Resistant starch		
	CE	SE	P-value	CE	SE	P-value	CE	SE	P-value
Intercept	66.58	0.26		5.60	0.05		1.34	0.01	
A-Temperature	6.39	0.58	*< 0.001	-1.57	0.13	*< 0.001	0.14	0.01	*< 0.001
B-Time	0.49	0.59	0.420	-0.04	0.13	0.731	0.01	0.01	0.710
AB	1.65	0.99	0.126	-0.40	0.22	0.092	0.03	0.02	0.258
A^2	2.72	1.03	*0.022	-0.84	0.22	*0.003	0.03	0.02	0.284
B^2	1.35	0.78	0.111	-0.37	0.17	0.051	0.03	0.02	0.087

Model terms with P<0.05 are significant and are assigned an asterisk (*); A: Temperature; B: Time; CE: coefficient estimate and SE: standard error.

In vitro protein digestibility and trypsin inhibitor activity

High drying temperature and long drying time resulted in high protein digestibility and low trypsin inhibitor activity (Fig. 2). R^2 values imply that 97.3% and 97.9% of variation in protein digestibility (equation 9) and trypsin inhibitor activity (equation 10) was attributed to the independent variables, temperature (A) and drying time (B).

$$\text{Protein digestibility (\%)}_{\text{Seeds}} = 66.58 + 6.39A + 0.49B + 1.65AB + 2.72A^2 + 1.35B^2 \text{ (model P value} < 0.001, R^2 = 0.973, \text{lack of fit P value } 0.169) \quad (9)$$

$$\text{Trypsin inhibitor activity (mg/g Protein DMB)}_{\text{seeds}} = 5.60\text{-}1.57A\text{-}0.04B\text{-}0.40AB\text{-}0.84A^2\text{-}0.37B^2 \text{ (model P value} < 0.001, R^2 = 0.979, \text{lack of fit P value } 0.362) \quad (10)$$

For both *in vitro* protein digestibility and trypsin inhibitor activity, temperature was the only significant variable (Table 5). There was a steep increase in *in vitro* protein digestibility (Figure 2A) and reduction in trypsin inhibitor activity as drying temperature increased from 60 to 80°C. In the temperature ranges of 40 to 60°C and 60 to 80°C, trypsin inhibitor activity reduced by 1.02% and 2.14% respectively with the steepest increase between 70 and 80°C (Fig. 2B). The observed pattern is in agreement with Damodaran (1996) who reported that during denaturation of most globular proteins, the monitored parameter remains fairly unchanged until the critical point of the denaturant is reached and then the parameter changes

abruptly. Roychaudhuri et al. (2003) also reported soybean Kunitz trypsin inhibitor to get denatured at approximately 65°C. Protein digestibility increased by 3.82% and by 7.99% as temperature increased from 40 to 60°C and 60 to 80°C, respectively (Fig. 2A). The steep increase between 60 to 80°C can be attributed to denaturation of the pumpkin seed proteins and the trypsin inhibitor since denaturation is accompanied by increased digestibility (Damodaran, 1996). The protein digestibility of the raw seeds (62.59%±0.15) was however lower than the values of 72% observed in seeds of fluted pumpkin (Fagbemi et al., 2005).

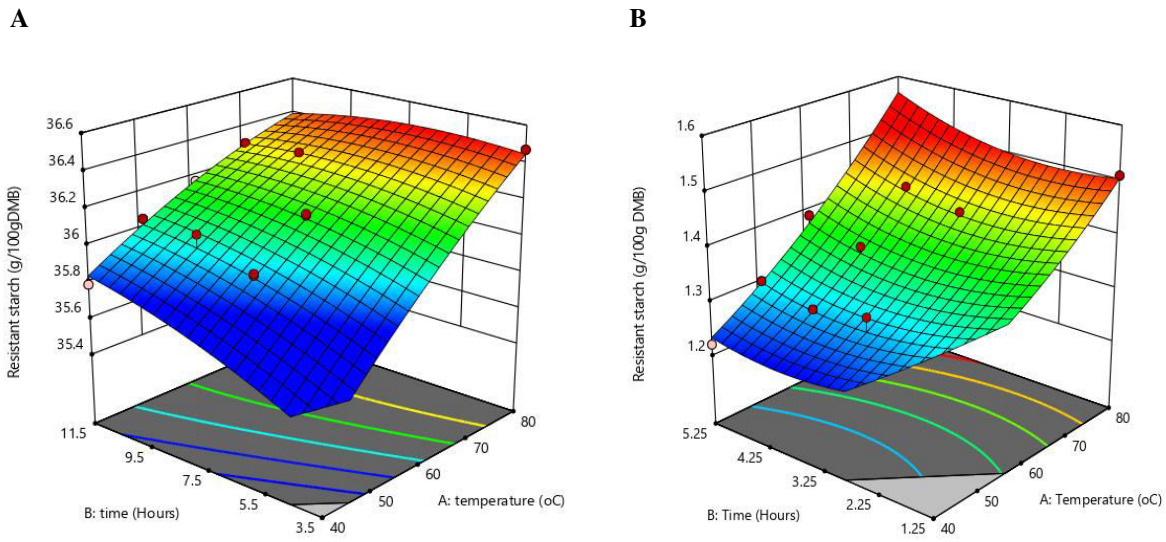

Fig. 1. Resistant starch content (%) of pumpkin flesh (A) and seeds (B) at different drying regimen.

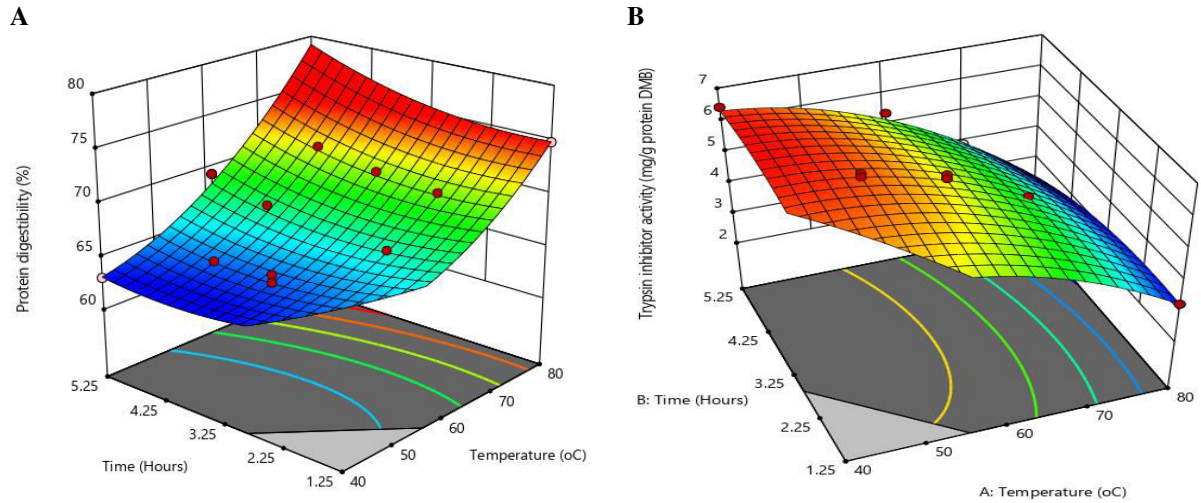

Fig. 2. *In vitro* protein digestibility and trypsin inhibitor activity and of pumpkin seeds at different drying regimen.

Ascorbic acid content of the flesh and seeds

R^2 values imply that 91.1% and 89.0% of the observed variation in ascorbic acid of the flesh (equation 11) and seeds (equation 12) was attributed to the independent variables, temperature (A) and drying time (B).

$$\text{Ascorbic acid (mg/100gDMB)}_{\text{flesh}} = 43.46 + 2.82A - 5.26B - 0.89AB - 12.61A^2 + 2.43B^2 (\text{model p value } < 0.001, R^2 = 0.911, \text{lack of fit P value } 0.182) \quad (11)$$

$$\text{Ascorbic acid (mg/100g DMB)}_{\text{Seeds}} = 14.42\text{-}0.24A\text{-}0.21B + 0.29AB\text{-}0.29A^2\text{-}0.11B^2 (\text{model P value} < 0.001, R^2 = 0.890, \text{lack of fit P value } 0.21) \quad (12)$$

Ascorbic acid content of the flesh was highest at drying conditions of 60°C: 5.5 hours (Figure 3A) whereas that of seeds was highest at drying conditions of 60°C: 2.25 hours (Fig. 3B). The quadratic temperature related term A^2 (mostly) and time, significantly and negatively affected the ascorbic acid content of the flesh (Table 4). For the seeds however, both temperature and time were significant and negatively affected the ascorbic acid content (Table 6). For both the flesh and seeds, ascorbic acid content was low at drying temperatures below and above 60°C. It was however lowest at temperatures below 60°C for the flesh, ascribed to the long period samples were exposed to the drying heat coupled with high activity of the enzyme ascorbic acid oxidase. Ascorbic acid oxidase activity increases upon damaging tissues (Gregory, 1996) for example during cutting of the pumpkin flesh into small pieces to be dried and it has optimum activity at 40°C.

Total carotenoids content of the flesh and seeds

R^2 values imply that 90.1% and 93.3% of sample variation for total carotenoids content of the flesh (equation 13) and seeds (equation 14) was attributed to the independent variables, temperature (A) and drying time (B).

$$\text{Total carotenoids (mg/100gDMB)}_{\text{flesh}} = 11.57 + 1.15A - 1.13B + 0.29AB - 2.53A^2 + 0.45B^2 (\text{model P value} < 0.001, R^2 = 0.901, \text{lack of fit P value } 0.132) \quad (13)$$

$$\text{Total carotenoids (mg/100g DMB)}_{\text{Seeds}} = 7.00 + 0.37A\text{-}0.01B + 0.19AB\text{-}0.23A^2 + 0.20B^2\text{-}0.18A^2B\text{-}0.16AB^2\text{-}0.58A^3\text{-}0.15B^3 (\text{model P value}0.002, R^2 = 0.933, \text{lack of fit P value } 0.988) \quad (14)$$

For both the flesh and seeds, temperature positively influenced the total carotenoids content, implying that an increase in drying temperature led to increased retention of total carotenoids (Fig. 4). Potosí-Calvache et al. (2017) also reported an increase in carotenoids retained in *Cucurbita moschata* as drying temperature increased from 45 to 55°C followed by a decline as drying temperature increased from 55 to 65°C. The observed carotenoid retention at high drying temperatures was probably attributed to the less drying time associated with high temperatures and the inactivation of oxidative enzymes. Besides Rodriguez-Amaya and Kimura (2004) reported degradation of carotenoids by oxidative enzymes to be more of a problem than thermal decomposition during processing and that rapid processing at high temperature was a good alternative.

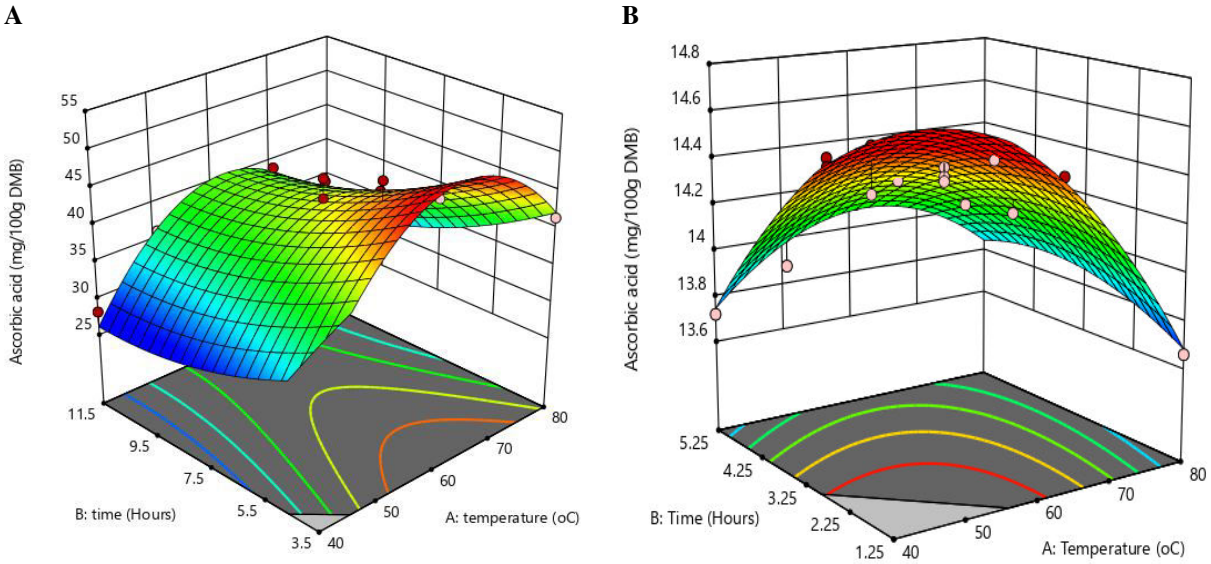

Fig. 3. Ascorbic acid content of pumpkin flesh (A) and seeds (B) at different drying regimen.

Table 6. Significance of the bioactivity parametric model terms for the dried pumpkin seeds

		Factor									
		Intercept	A	B	AB	A^2	B^2	A^2B	AB^2	A^3	B^3
TAA	CE	44.87	2.17	0.04	0.85	-1.59	0.84	-0.66	-0.81	-2.77	-0.71
	SE	0.14	0.45	0.42	1.13	0.81	0.85	1.59	1.25	1.62	0.91
	P-value		*0.001	0.918	0.472	0.091	0.355	0.689	0.536	0.132	0.459
AA	CE	14.42	-0.24	-0.21	0.29	-0.29	-0.11				
	SE	0.04	0.08	0.09	0.14	0.15	0.11				
	P-value		*0.015	*0.031	0.068	0.082	0.338				
TC	CE	7.00	0.37	-0.01	0.19	-0.23	0.20	-0.18	-0.16	-0.58	-0.15
	SE	0.02	0.07	0.06	0.18	0.13	0.14	0.26	0.20	0.27	0.15
	P-value		*0.001	0.889	0.324	0.123	0.196	0.514	0.454	0.064	0.345
AT	CE	6.47	-0.62	-0.21							
	SE	0.03	0.07	0.06							
	P-value		*< 0.001	*0.006							
BT	CE	2.90	-0.18	-0.09							
	SE	0.01	0.02	0.02							
	P-value		*< 0.001	* 0.001							
AT^*	CE	3.35	-0.18	-0.09							
	SE	0.01	0.02	0.01							
	P-value		*< 0.001	*< 0.001							

Model terms with P<0.05 are significant and are assigned an asterisk (*); A: Temperature; B: Time; CE: coefficient estimate and SE: standard error. (Total antioxidant activity: TAA; Ascorbic acid: AA; Total carotenoids: TC; Alpha tocopherol: AT; Beta tocopherol: BT; and Alpha tocotrienol: AT^*.)

Tocopherols and tocotrienol contents of the seed samples

R^2 values imply that 83.9%, 87.1% and 87.4% of sample variation for alpha tocopherol (equation 15), beta tocopherol (equation 16) and alpha tocotrienol (equation 17) was attributed to the independent variables, temperature (A) and drying time (B).

$$\alpha - \text{Tocopherol (mg/100gDMB)}_{\text{Seeds}} = 6.470 - 0.62A - 0.20B \text{ (model P value} < 0.001, R^2 = 0.839, \text{lack of fit P value } 0.498) \quad (15)$$

$$\beta - \text{Tocopherol (mg/100gDMB)}_{\text{Seeds}} = 2.90 - 0.18A - 0.08B \text{ (model P value} < 0.001, R^2 = 0.871, \text{lack of fit P value } 0.498) \quad (16)$$

$$\alpha - \text{Tocotrienol (mg/100gDMB)}_{\text{Seeds}} = 3.35 - 0.18A - 0.08B \text{ (model P value} < 0.001, R^2 = 0.874, \text{lack of fit P value } 0.517) \quad (17)$$

Temperature and time, significantly and negatively influenced the alpha tocopherol, beta tocopherol and alpha tocotrienol content (Table 6) implying that increasing the drying temperature led to more reduction in tocopherols and tocotrienol, graphically presented in the 3D response surface plots (Fig. 5). Their reduction can be explained by the fact that thermal induced degradation of vitamin E increases with increase in temperature (Gregory, 1996).

Total antioxidant activity

R^2 values imply that 90.7% and 93.5% of sample variation for total antioxidant activity of the flesh (equation 18) and seeds (equation 19) was attributed to the independent variables, temperature (A) and drying time (B).

$$\text{TAA(\% DPPH scavenged)}_{\text{Flesh}} = 35.22 + 2.63A\text{-}6.97B\text{-}4.84AB\text{-}7.82A^2\text{-}1.74B^2 + 0.33\,A^2B + 4.13AB^2\text{-}6.43A^3 + 3.95B^3 \text{(model P value } 0.006, R^2 = 0.907, \text{lack of fit P value } 0.06) \quad (18)$$

$$\text{TAA(\% DPPH scavenged)}_{\text{Seeds}} = 44.87 + 2.17A + 0.04B + 0.85AB - 1.59A^2 + 0.84B^2 - 0.66A^2B - 0.811AB^2 - 2.77A^3 - 0.71B^3 \text{ (model P value } 0.002, R^2 = 0.935, \text{lack of fit P value } 0.955) \quad (19)$$

The 3D plot (Figure 6) showed an increase in retention of total antioxidant activity as drying temperatures increased from 40 to 60 °C (flesh) and 40 to 70°C (seeds) after which total antioxidant activity reduced with further increase in drying temperature. Time significantly and negatively affected the total antioxidant activity of the flesh (Table 4) probably because ascorbic acid, which was the dominant antioxidant, was significantly and negatively affected by time. In addition, ascorbic acid as an antioxidant is known for regenerating other antioxidants like tocopherols. Besides, total antioxidant depends on synergistic and redox interactions among the different molecules, in addition to the levels of antioxidants in food (Pellegrini et al., 2003). However, for the seeds, temperature was the most influential and positively affected the total antioxidant activity (Table 6). Ekorong et al. (2015) also found the effect of drying temperature to outweigh the effect of time on the total antioxidant activity of dried mango seed kernels.

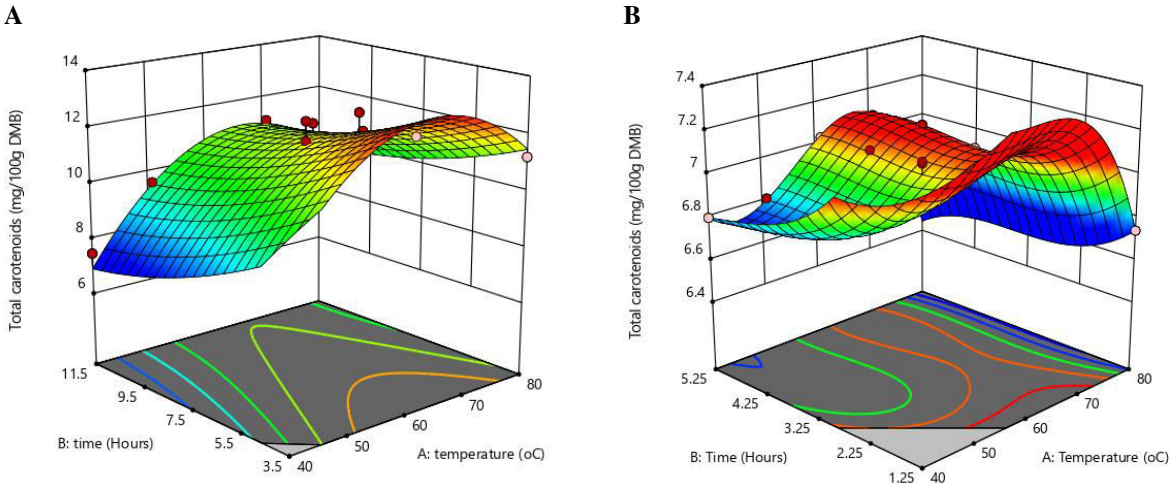

Fig. 4. Total carotenoids of pumpkin flesh (A) and seeds (B) at different drying regimen.

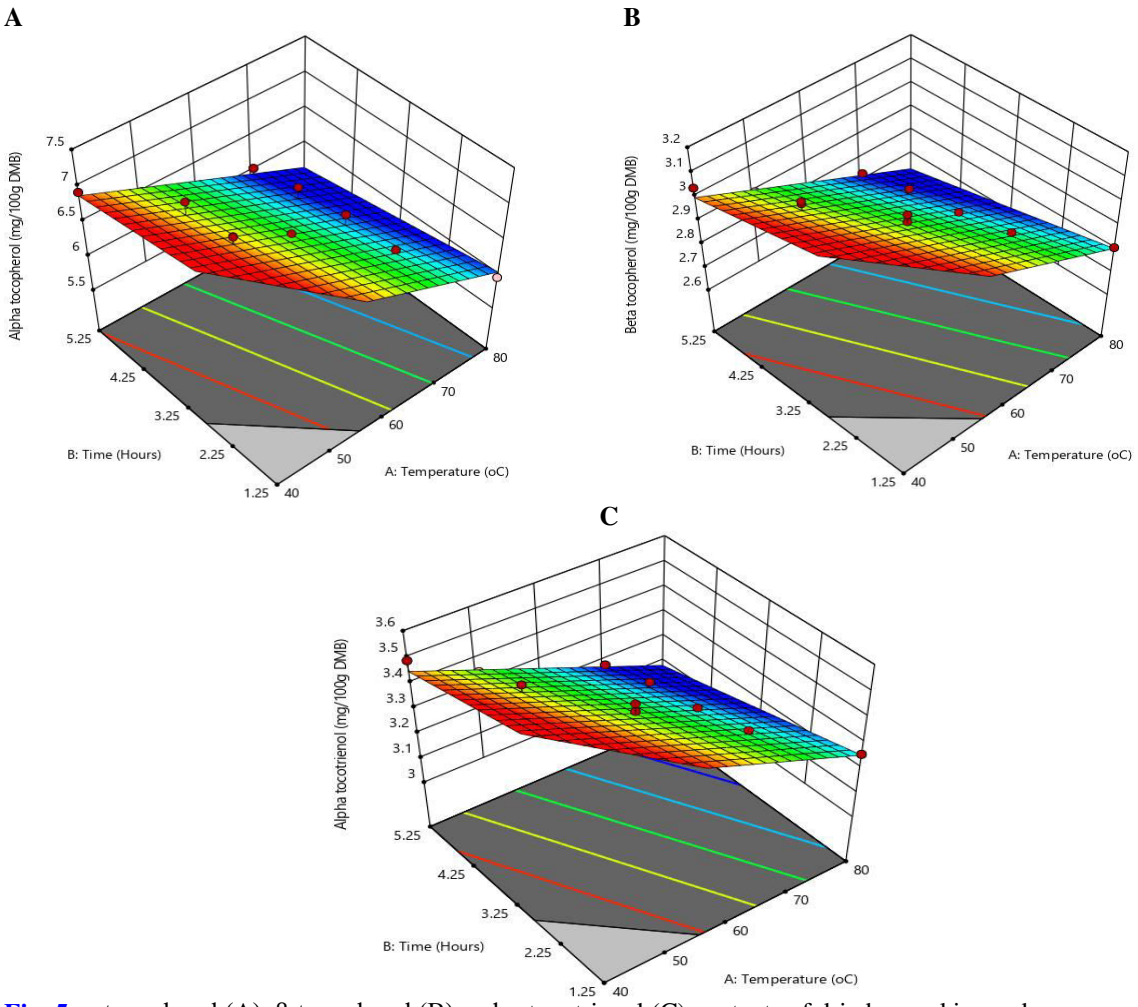

Fig. 5. α-tocopherol (A), β-tocopherol (B) and α-tocotrienol (C) contents of dried pumpkin seeds.

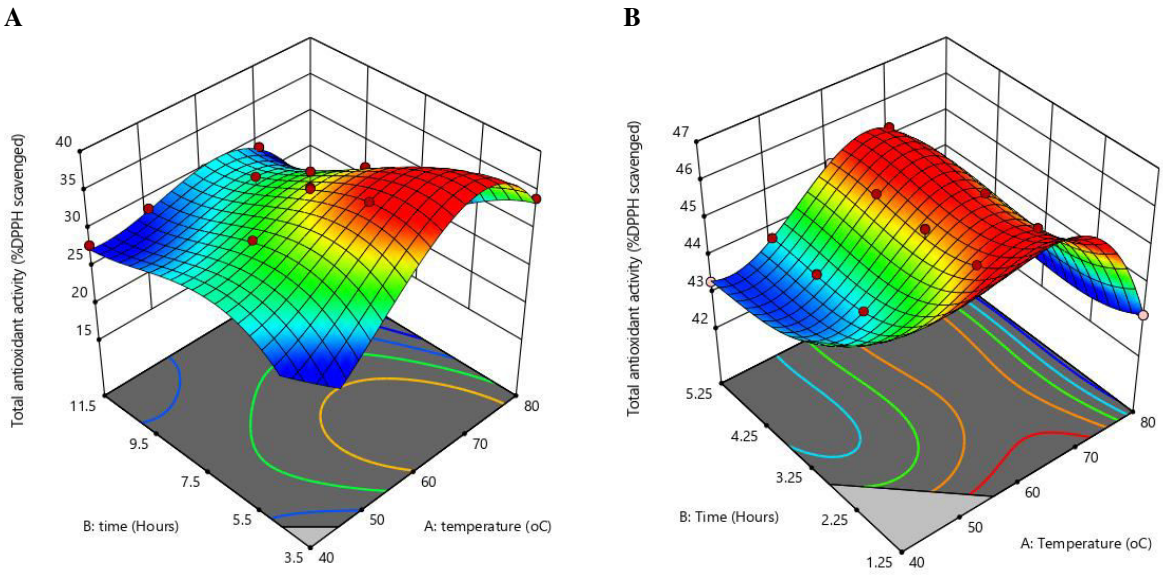

Fig. 6. Total antioxidant activity of pumpkin flesh (A) and seeds (B) at different drying regimen.

CONCLUSION

The increase in resistant starch content at the optimised drying conditions 57°C; 6.9 hours (flesh) implies better nutritional quality and nutraceutical value of flour from flesh especially in conferring health benefits like reducing the chances of developing Type-2 diabetes mellitus, obesity, and cardiovascular diseases. Increase in *in vitro* protein digestibility and reduction in trypsin inhibitor activity of seeds at the optimised drying conditions of 60°C; 3.15 hours implies that the body will utilise better the protein of the pumpkin seed flour. In addition, the body shall obtain maximum cumulative capacity of the components in flours to scavenge free radicals thus delaying the onset and progression of diseases like cancer.

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Conflicts of interest
The authors have no conflict of interest.

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