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Production of high-quality flour and the made biscuits from Pumpkin

Nguyen Van Toan[1*], Nguyen Thi Thanh Thuy[2]
[1, 2] Department of Food Technology, School of Biotechnology, International University, Vietnam National University, Ho Chi Minh City, Block 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

Abstract
Pumpkins provide substantial amounts of alpha-carotene, beta-carotene, lutein and zeaxanthin, which effectively makes them an excellent source of antioxidants. Pumpkin also offers healthy amounts of fiber, potassium, riboflavin, vitamin C, and iron. In addition, pumpkin is a good, low-fat source of vitamin E. Then as a consequence, a successful combination of pumpkin flour with wheat flour for biscuits production would be nutritionally advantageous. The composite flour from Wheat flour and Pumpkin flour of variety TLP368 for production of biscuits was incorporated with ratio of 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100, respectively. The physico-chemical analysis and sensory evaluation were done to know the acceptability of developed biscuits. On the basis of nutritional value, biscuits containing 40% and 60% pumpkin flour had significantly higher ash, fiber, protein than those the control samples. At the same time, the sensory evaluation brought the scores of biscuits containing 40% and 60% pumpkin flour which were significantly higher than those of the control biscuits in all attributes, respectively. Biscuits with the addition of pumpkin flour enhanced the yellow color and level of beta- carotene content in the food. The obtained results of this study have indicated that the developed biscuits were not only improved in terms of nutritional value and health benefits, but also had high potential of being accepted by consumers.

Keywords: pumpkin flour, pumpkin biscuits, composite flour, physical properties, beta-carotene, sensory evaluation

Introduction

Pumpkins is native to North America and it is extensively grown all around the world. Pumpkin belong to the genus Cucurbita of the family Cucurbitaceae. Especially, there are three typical types of pumpkin in the world that is Cucurbita pepo, Cucurbita maxima and Cucurbita moschata [1]. Pumpkins vary greatly in shape, size and colors. In terms of colors it comes in either orange or yellow. In addition, some varieties may be dark to light green, brown, white, red and gray. The thick shell of pumpkin contain seeds and pulp. In composition, fruit pulp has golden-yellow to orange color.

In Vietnam, Pumpkin is grown year-round, it is well adapted to hot and humid tropical climates A biscuit is a kind of cake which is small flat, crisp and made from flour [2]

Over the past few years, the confectionery industry in Vietnam has had a high and stable growth rate. The average annual revenue growth rate of the confectionary industry in 2006-2014 was 25%, It has been forecasted that from 2015 to 2019 the growth rate will be around 8-9%, at least (Agrofood Research Report, EU-Vietnam Business Network, STINFO 12-2015).

Biscuits have been one of the oldest baked goods and consumed extensively all over the world by all age groups. The popularity of biscuits comes from their attributes such as high palatable, dense nutrients, quickly released energy and available in convenient sizes as well as in various forms. In addition, the biscuits formulation can be modified easily to meet the nutritional demands of the target consumers (Ashaye, Olanipekun *et al*. 2015). Since biscuits are dried to low moisture content, and this can ensure their long shelf life storage, and especially free from microbial spoilage (Okaka 2005). Being faster in the growth of the biscuits manufacturing, there is a huge scope of research on diversification of this baked product in Vietnam (Agrofood Research Report, EU- Vietnam Business Network, STINFO 12- 2015).

It has been claimed that the loaf volume and organoleptic acceptability of wheat bread were enhanced by adding a certain mount of pumpkin powder [3]. According to Lee *et al* [4] stated that stated that by adding certain amount of pumpkin powder the Beta-Carotene content in noodles was clearly enhanced. Ptitchkina [3] used the pumpkin powder in bread making at different levels (5-10 g) of pumpkin powder and reported the enhancement of loaf volume and sensory ratings.

Pumpkin flour is a good source of food containing high and healthy amount of dietary fiber [5]. Moreover, Pumpkin contains high content of Beta carotene, pectin, some vitamin, material salts, etc. Pumpkin also included the various source of carotenoids and ascorbic acid [6] which have important roles in nutrition as provitamin A (antioxidant). Nevertheless, α, ß-carotenes, lutein, cryptoxanthin and zeaxanthin, etc. as natural poly-phenolic flavonoid compounds in pumpkin.

According to research, they claim that foods include high level of Beta-carotene that may reduce the risk of cancer, protect heart, and delay aging and body degeneration [7]. Beta-Carotene may help lower your risk of metabolic syndrome [8], may protect your skin from the sun's damaging UV rays [9]. Vitamin-A is known as a strong natural antioxidant and it is necessary for preserve the integrity of skin and mucosa and also as an essential vitamin for good eyesight. Heinonen and

Albanes [10] suggested human body can be protected against lung and oral cavity cancers by increasing vitamin-A in the body. Zeaxanthin is an active biological compounds found in Pumpkin which is a natural anti-oxidant included with ultra-violet rays that gives sieving actions in the macula lutea in the retina of the eyes [11]. Thus, it can protect eyes of the older adults. Besides, Pumpkins also provide lots of riboflavin, potassium, copper, manganese and smaller significant amounts of niacin, folate, phosphorus, andiron, etc. The fiber, potassium, and vitamin B6, vitamin C content in pumpkin support well for heart [12].

Although many studies on the evaluation and use of pumpkin have been conducted as mentioned above, there is no information on how to prepare and improve the quality of flour and the made biscuits from pumpkin. So, it is scientifically and economically important to know whether the made flour from Pumpkin can be used for the production of high quality biscuits with improved nutritional values. Also, consequence of various proportion of Pumpkin to wheat flour in biscuits formation needs to be determined in order to make high quality final biscuits products. In summary, this study has been conducted to determine the suitable process for preparation and possibly improved quality production of flour and the made biscuits from the Pumpkin.

Materials and Methods

Research object and location

30 kg pumpkin of variety TLP368 without any bruises were procured from local market in Ho Chi Minh City.

Fig 1: Pumpkin of variety TLP368 in this study

Other major ingredients needed to prepare for biscuits production such as wheat flour, sugar, baking powder, and butter were obtained from Nhat Huong Company, Ho Chi Minh City, Vietnam. Chemicals used for sample analysis such as hexane, sodium hydroxide, etc. were purchased from Bach Khoa Limited Company in Vietnam.

The experimental studies were carried out in the laboratories of Food technology Department, School of Biotechnology, International University – Vietnam National University in Ho Chi Minh City.

Preparation of Pumpkin flour

Fresh pumpkins were be washed, trimmed and peeled to make them free from soil, rotting or insect damage. The pumpkin pieces were then cut into slices before being steamed for 10 minutes. The pumpkin cubes were mashed, spread evenly on different trays, and then dried in an oven at 55°C for 24 hours. The dry samples were milled into flour using the laboratory grinder and passed through 250 µm sieve to obtain uniform sized flour. The flour was then packed in sealed plastic bag and stored at ambient temperature till further used.

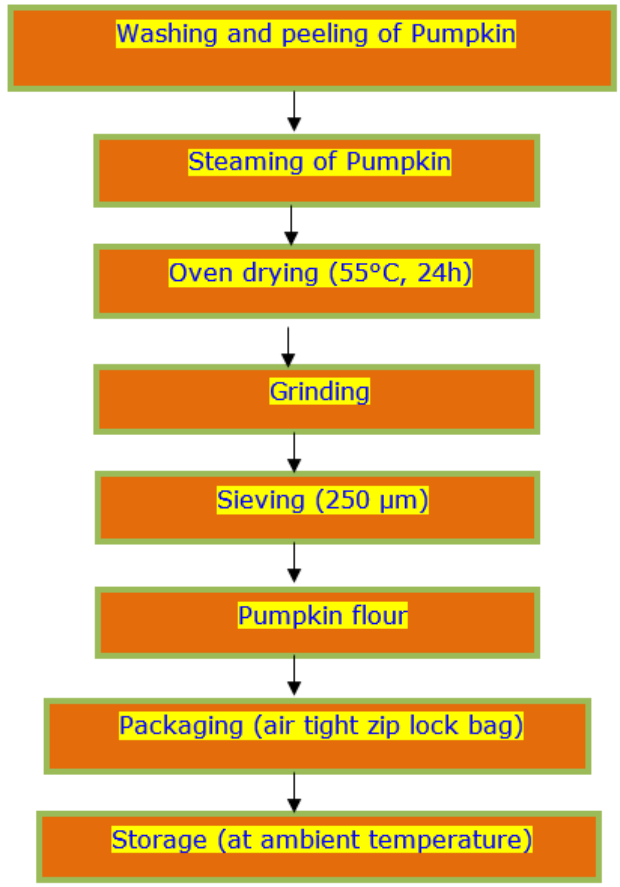

Fig 2: Flow chart for the preparation of pumpkin flour

Preparation of Wheat- Pumpkin biscuits

The Wheat- Pumpkin flour composites were prepared at different ratios (of 100:0; 90:10; 80:20; 70:30; 60:40; and 50:50) with other ingredients were weighed accurately as the formulations shown in Table 1. Shortening and sugar were creamed in a mixer before the homogenized mixture of dried ingredients was added. Smooth dough was formed and rolled to a 3-5mm sheeted size with the help of a rolling pin. A round cutter of 4cm diameter was used to create a uniform shape for all biscuits. Then, they were transferred to a lightly greased baking tray and baked at 150°C for 10 minutes in a preheated oven. After baking, all biscuits were allowed to be cool completely (about 30 minutes) and stored in air tight containers for 12 hours before further analysis.

As shown in the all well prepared tables, A is symbol marked biscuits produced from 100% wheat flour. Similarly, B is marked biscuits produced from 90% wheat and 10% Pumpkin flour, C is marked biscuits produced from 80% wheat and 20% Pumpkin flour, D is marked biscuits produced from 70%

wheat and 30% Pumpkin flour, E is marked biscuits produced from 60% wheat and 50% Pumpkin flour, and F is marked biscuits produced from 60% wheat and 50% Pumpkin flour.
A = 100: 0 ratio of wheat- pumpkin flour in biscuits
B = 80: 20 ratio of wheat- pumpkin flour in biscuits
C = 60: 40 ratio of wheat- pumpkin flour in biscuits
D = 40: 60 ratio of wheat- pumpkin flour in biscuits
E = 20: 80 ratio of wheat- pumpkin flour in biscuits
F = 0: 100 ratio of wheat- pumpkin flour in biscuits

Table 1: Ingredients used in the preparation of biscuits

Ingredients	Samples (g)					
	A	B	C	D	E	F
Wheat flour	100	80	60	40	20	0
Pumpkin flour	0	20	40	60	80	100
Powdered sugar	15	15	15	15	15	15
Butter	40	40	40	40	40	40
Baking powder	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5

Preparation of composite flour (wheat flour: Pumpkin flour) at different ratios

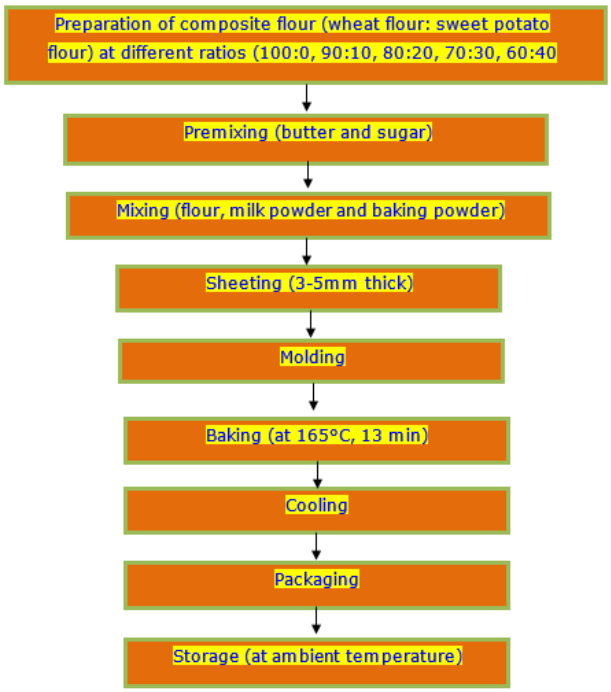

Fig 3: Flow chart for the preparation of biscuits

Proximate analysis of Pumpkin flour
The proximate analysis of the composite flours and biscuits moisture, protein, ash, fiber and fat content were determined to the methods described by A.O.A.C (2012). Total carbohydrates were calculated by difference (AOAC, 1990).
Moisture content: The flour sample (3g) was taken in a pre-weighed porcelain crucible, was dried to constant weight at 105°C for 14 hours. Loss in weight was taken as the moisture content of the sample (AOAC, 2012).

% Moisture = Weight loss (g) x 100 / sample weight (g)

Ash content
The crucibles containing 5g of sample was charred on a heater before kept in the muffle furnace at 550oC for 4 hours until only white matters can be seen. Then, the crucible with ash content was then cooled in a desiccator and weighed accurately to a constant weight (AOAC, 2012).

Fat
Fat content was determined by extracting 3g of sample with hexane using Soxhlet apparatus for 6 hours. The residual hexane was removed from the extracted sample by evaporation. The extracted fat was then dried and weighed (AOAC, 2012).

Protein
Protein content was analyzed by using the Kjeldahl method according to the AOAC methods (2012). 1g of sample was placed in a digestion tube; 0.2g $CuSO_4$, 1g K_2SO_4, and 20ml concentrated H_2SO_4 were added to the tube with sweet potato flour. The sample was let digested on digestion block until white fumes can be seen and continue heated for about 60 – 90 minutes until cleared with no charred material remaining. Tube was placed in the distillation apparatus and 50ml NaOH 32% was added. The ammonia in the sample was steam-distilled for 5 minutes into a receiving flash containing 4% boric acid. The sample was titrated with H_2SO_4 0.1N solution. The protein was calculated by the equation: %Nitrogen x 6.25.

Crude fiber
Crude fiber was determined following the approved AOAC method 962.09. Crude fiber is loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions.
2g of each sample was extracted with ether or petroleum ether and transferred to beakers of ceramic fiber mixture. Two beakers of ceramic fiber mixture for each sample were prepared as follows: 1.5 g dry weight of sample was added to each 100 ml beaker, then 60-75 ml 0.255N H_2SO_4 was added to each beaker and allowed to soak. Beakers were placed on digestion apparatus with pre-adjusted hot plate and boiled exactly 30 minutes. Contents of beaker were filtered through Buchner funnel (pre-coated with ceramic fiber if extremely fine materials are being analyzed). Beaker was rinsed with 50-75 ml boiling H_2O and washed through Buchner funnel. Residue was removed before 200 ml 1.25% NaOH was added and boiled exactly 30 minutes. Contents was filtered and then washed with 25 ml boiling 1.25% H_2SO_4, 50 ml H_2O and 25 ml alcohol. Residue was transfer to ashing dish, dried for 2 hours at 130 ± 2° C. Then, it was cooled in desiccator and weighed. Residue was ignited 30 minutes at 600 ± 15°C and cooled in desiccator before being reweighed.
% Crude fiber in ground sample = C = (Loss in weight on ignition loss in weight of ceramic fiber blank) x 100- weight sample

Carbohydrate
Total carbohydrate was determined by the difference [13]

% Carbohydrate = 100 – % (protein+ fat + ash+ fiber + moisture)

Functional properties analysis of the flour samples

Bulk density: Bulk density was determined following the method described by Eleazu and Ironua (2013) [14] and Onabanjo and Dickson (2014). A (10ml) graduated cylinder, previously tarred, was gently filled with 5g of sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was to a constant. The bulk density of the sample (g/ml) was calculated as weight of the sample per unit volume of sample.

Water Absorption Capacity (WAC): The WAC of the sample was determined using the method as described by Eleazu and Ironua (2013) [14] and Onabanjo and Dickson (2014) [15] with minor modification. A measured quantity (1g) of the sample was dispersed in 10 ml of distilled water in a conical graduated centrifuge tube. The sample was thoroughly mixed for 30 seconds and allowed to stand at room temperature for 30 minutes before being centrifuged at 4000 rpm for another 20 minutes. The volume of the supernatant was measured directly from the graduated centrifuge tube. The amount of the absorbed water was multiplied by the density of water (1 g/ml) and results were expressed as g/100 g.

Oil Absorption Capacity (OAC): Oil absorption capacity of the flour was determined using the method as described by (Adepeju, Gbadamosi *et al*. 2011) and (Eleazu and Ironua 2013). One gram of sample was mixed with 10ml of pure canola oil for 60 seconds. The mixture was set to stand for 10 minutes at room temperature, centrifuged at 4000rpm for 30 minutes and the oil that separated was carefully decanted. The tubes were allowed to drain at an angle of 45°C for 10 minutes and then weighed. Oil absorption was expressed as percentage increase of the sample weight.

Proximate analysis of developed biscuits: The same methods used for pumpkin flour analysis were applied for determined the moisture, fiber, protein, ash, and fat content of biscuits.

Assay of Beta- carotene of pumpkin flour and developed biscuits

Following the method of Khalil and Varananis (1996) [16], they were calculated carotene by extracting from vegetables.

Extraction: 10g of sample was extracted by adding 30ml of acetone and then 0.1% (BHT) solution in acetone. The resulting extract was filtered. The residue was washed with acetone. This step repeated till it become colorless. After that, the filtrate was combined with 20gm of anhydrous sodium sulphate. The extract was transferred quantitatively to 100ml volumetric flask and the volume was made up to the mark with acetone and water.

Standard preparation for Beta- Carotene: Taking 10mg of Beta- Carotene in 100ml n-hexane to conduct stock solution. The concentration of stock solution was equal to 100 ppm. The stock solution was diluted to various known concentration respectively twenty, forty and sixty ppm. Perkin Elmer HPLC programmer including LC-1000 pump (Isocratic), which having C18 column and linked together with LC 250 UV/VIS detector. Using HPLC system to determine the peak identification and quantification. Wave length was fixed at 452 nm. The pressure of the column was kept 1800-2000 PSI.

Physical properties measurements of biscuits

The physical properties such as weight, width, spread ratio, volume, density and thickness were estimated. The width of biscuits was measured by placing 6 biscuits edge-to-edge to get the average value in millimeters. The thickness was measured by stacking 6 biscuits on top of each other to get the average value and recorded in millimeters. The spread ratio was defined as width divided by the thickness. Digital weighing scale was used to determine the weight (in grams) of biscuits. The area multiplied by thickness gave the volume of biscuits. After calculating volume, density was obtained by ratio of weight of volume [17, 18.19, 20, 21].

Sensory evaluation

The consumer acceptance of six different samples of biscuits was evaluated by fifty judges comprising undergraduate students of International University without training. The sensory evaluation test was conducted in the air-conditioned laboratory, which provided a quiet and comfortable environment. The biscuits were served on white disposable plastic trays and tap water was provided for rinsing. Samples were coded with different symbols and the sample order was randomized. Consumers were asked to evaluate the color, taste, flavor, texture and overall acceptability of the biscuits using a 5-point hedonic scale.

Statistical analysis

Data was subjected to analysis of variance using the "Statistical Package for Social Sciences" (SPSS) version 19.0. Results were presented as means ± standard deviations of triplicate experiments. Significant difference was established at $p \leq 0.05$.

Results and Discussion

Proximate analysis of pumpkin flour

The results of analysis proximate attributes of pumpkin flour are presented in Table 2.

Table 2: Proximate values of pumpkin flour

Components (%)	Value*
Moisture (d.w.b)	7.90 ± 0.11
Ash	4.51 ± 0.08
Fat	0.85 ± 0.01
Protein	7.63 ± 0.29
Crude fiber	4.78 ± 0.00
Total carbohydrate	74.33 ± 0.26

*Values in the table represent the means ± standard deviations (n = 3 replicates) DWB = dry weight basis

The table 2 shows percentages of moisture content of pumpkin flour (dry weight basis) was 7.9 ± 0.11%. Drying is one of the majorities commonly used methods for food preservation, and its play the major purpose is to remove water from the food to a level in which microbial spoilage and deterioration reactions are greatly minimized [22]. Moreover, besides the main purposes is providing longer shelf-life of pumpkin, it also

begins smaller space requirements for storage and lighter mass for shipping, it permits to maintain the nutritive value [23]. Data collected from many researches on pumpkin flour have shown that the moisture content of the flour is in the range of 1.80 to 10.96% [24, 25]

The mineral content of the food material was presented in ash content of food analyzed, it has long been identified to containing calcium (616.73mg/100g), phosphorus (817.88mg/100g), sodium (21.50mg/100g), potassium (5185.11mg /100g), iron, zinc and copper as they are the main mineral constituents contained in pumpkin flour [26].

Also, from the Table 2, the ash content of the flour was 4.51 ± 0.08%, of which the collected value in this study was in a higher acceptable range than the result of ash content in pumpkin powder (3.8%), which were estimated by Ptitchkina *et al*. (1998) [27] and lower than the results of Egbekun *et al*, of which was of 6.13%, while 5.37% of total ash content was the similar to the result which was investigated by Saeleaw & Schleining(2011) [28].

The fat content in fresh pumpkin pulp were low and around 1.26%, which was determined by Păucean & Man, 2014 [29]. The fat content of pumpkin flour in this study has valued of 0.85 ± 0.01% which was similar data collected by Saeleaw & Schleining (2011) [28].

It is clear that the percentage of protein content in pumpkin flour is 7.63 ± 0.29%. On the other hand, this result is similar With the data collected by Pongjanta, Naulbunrang, Kawngdang, Manon, & Thepjaikat, 2006 [30].

According to (Pongjanta *et al*., 2006) [30] the total crude fiber was 2.90 ±0.47% compare with the result obtained in this study was within that range of 4.78%. It is generally agreed that dietary fiber is an available tool in controlling oxidation in food products and as a functional food ingredient (Eleazu and Ironua 2013) [14]. Furthermore, dietary fiber reduces the absorption of cholesterol from the intestine, slows digestion and converts starch into simple sugars, an important factor in controlling diabetes [31]. One study reported that in a 20-month period, every day if increasing 1 gram of fiber were consumed, the body weight would be decreased 0.25 kg [30]

Scientifically and practically, Determining the total carbohydrate content of the studied food materials is significantly important. As shown in the Table 2, data on the total carbohydrate content was occupied high percentage of 74.33 ± 0.26%.

Functional properties of composite flour

For food processing, storage and food preparation, functional properties of foods are significant properties to establish the applications and uses of food material since they concern the general quality of foods. The important functional properties so as to be usually assayed include: water absorption capacity, bulk density, oil absorption capacity, etc. [32] The results of functional properties of pumpkin flour (PF) and the composite flour samples are as showed in Table 3.

Table 3: Effect of incorporating pumpkin flour on the functional properties of the composite flours

Sample	Bulk density (g/cm3)	Water absorption Capacity (g/g)	Oil absorption capacity (g/g)
A	0.69 ± 0.01^{b}	1.72 ± 0.09^{d}	2.76 ± 0.03^{a}
B	0.70 ± 0.03^{b}	2.52 ± 0.07^{c}	2.72 ± 0.02^{ab}
C	0.72 ± 0.03^{b}	2.77 ± 0.02^{b}	2.69 ± 0.03^{ab}
D	0.73 ± 0.03^{b}	2.84 ± 0.13^{b}	2.68 ± 0.03^{ab}
E	0.74 ± 0.03^{b}	2.88 ± 0.03^{b}	2.64 ± 0.03^{b}
F	0.91 ± 0.02^{a}	3.32 ± 0.01^{a}	2.42 ± 0.07^{c}

*Values in the table represent the means ± standard deviations (n = 3 replicates) The values denoted by different letters in the same column are significantly different ($p \leq 0.05$)

Bulk density

The result of bulk density for wheat flour (sample A) was 0.69 ± 0.01 g/cm3 while pumpkin flour (sample F) recorded as 0.91 g/cm3. It can be clearly seen from table 3, when more and more pumpkin flour was incorporated into wheat flour, the bulk density of composite flour was decreased. The values for the samples ranged between 0.69 to 0.91 g/cm3, with sample F (100% of pumpkin flour) recorded the highest value and the lowest was sample A (100% of wheat flour). The values of bulk density among studied samples were reported insignificantly different ($p>0.05$).

Scientifically and economically, bulk density is an important factor, second only to moisture. The bulk density also content generally affected by the particle size and density of the flour and so, it is a really important approach to determining the type of packaging material required, material handling and application in wet processing in the food industry (Adeleke & Adedeji [31], 2010; Onabanjo & Dickson, 2014 [15]). Henceforward, when the particle size was increased, bulk density was decreased. Consequently, enhancement in bulk density is really necessary as it takes advantage in package processing, then a greater quantity may be packed within a constant volume (Eleazu & Ironua, 2013) [14].

Water Absorption Capacity (WAC)

Water absorption is a capacity of a product to link with water under conditions where water is limiting such as doughs and pastes. During the Table 3, there were significant differences ($p<0.05$) in the WAC of remain flours comparing with the control sample A. However, the results were similar among sample C, D and E. The result ranged between 172 to 332% for all samples, with sample F (100% of pumpkin flour) recorded the highest value and the lowest was sample A (100% of wheat flour). This suggested that when more pumpkin flour was added to wheat flour, the WAC of the blended samples was increased.

The ability of flour to absorb water is called as water absorption capacity. Iwe and Onalope (2001) [34] mentioned that high digestibility of the starch is based on increase in WAC. In food system, the feature of WAC is to improve yield, consistency of food (Eleazu & Ironua, 2013) [14]. WAC always associated with the amylose leaching, solubility and

loss of starch crystalline structure, so increasing WAC that leads to increasing those (Chandra, Singh, & Kumari, 2015) [35]. This also leads to the weakened dough, decreases its stability and extensibility. On the other hand, pumpkin flour composites can be used in making biscuit

Oil Absorption Capacity (OAC)

Data shown in the Table 3 indicated that the results of the study recorded significant difference ($p< 0.05$) in OAC from sample A to sample F. The flour derived from this pumpkin flour variety had considerable oil absorption capacity- OAC (242%). Among all composite flour samples, sample A recorded the highest value in OAC (276%) and the sample E recorded the least value (272%). The OAC of flour composites slightly decreased as more and more pumpkin flour was incorporated, which indicated diluting effect of pumpkin flour on OAC of wheat flour.

According to the observation of Kinsella and Melachour [36], the oil absorption capacity interrelated to the ability of proteins to attach fat is important property in food formulations since fats be active as flavor retainer and improve the mouth feel of foods.

Physical properties of developed biscuits

Statistics on the physical estimations of biscuits made from wheat flour and composite flour with several ratios of pumpkin flour are presented in Table 4.

Table 4: Physical properties of developed biscuits

Sample	Width (cm)	Thickness (cm)	Spread ratio	Weight (g)	Volume (cm^3)	Density (g/cm^3)
A	5.07 ± 0.01^{a}	0.45 ± 0.01^{a}	11.19 ± 0.15^{d}	7.62 ± 0.05^{a}	9.16 ± 0.11^{a}	0.83 ± 0.01^{d}
B	5.05 ± 0.02^{ab}	0.43 ± 0.01^{b}	11.67 ± 0.29^{cd}	7.42 ± 0.07^{b}	8.69 ± 0.28^{b}	0.86 ± 0.02^{cd}
C	5.04 ± 0.01^{bc}	0.41 ± 0.01^{c}	12.19 ± 0.16^{c}	7.25 ± 0.05^{c}	8.23 ± 0.12^{c}	0.88 ± 0.01^{c}
D	5.03 ± 0.02^{bc}	0.39 ± 0.01^{d}	12.80 ± 0.23^{b}	7.24 ± 0.02^{c}	7.82 ± 0.05^{d}	0.93 ± 0.01^{b}
E	5.02 ± 0.02^{c}	0.38 ± 0.01^{de}	13.09 ± 0.23^{ab}	7.17 ± 0.02^{cd}	7.57 ± 0.07^{de}	0.95 ± 0.01^{ab}
F	5.01 ± 0.01^{c}	0.37 ± 0.01^{e}	13.66 ± 0.23^{a}	7.07 ± 0.01^{d}	7.21 ± 0.11^{e}	0.98 ± 0.02^{a}

*Values in the table represent the means ± standard deviations (n = 3 replicates).

The values denoted by different letters in the same column are significantly different ($p \leq 0.05$)

The spread ratio is an important aspect that affected to quality. Jothi, Hashem, Rana, Rahman, and Shams-Ud-Din (2014) [17] stated that the spread ratio is the important factor that affected to quality parameters of biscuits since it correlated with texture, grain finesse, bite and overall mouth feel of the biscuits. Figure 4 shows that the level of composite flour affect to the spread ratio of the final product. Especially, when the level of pumpkin flour was increased, the spread ratio of final product was increased. Sample F were recorded the highest value of spread ratio (13.66), while the lowest value belonged to sample A (11.19). An increase in pumpkin flour content significantly increased the spread ratio of the biscuits, which was directly related to their thickness, whereas the diameter was generally not affected. The study of Aziah, Noor, & Ho (2012) [37] and Miller, Hoseney, & Morris (1997) [36] revealed that the formation of continuous gluten web was affected by protein in the flour that increases the viscosity and stops the flow of the dough. In conclusion, the biscuits made from different ratios of pumpkin flour had higher spread ratio of than that of the wheat biscuits.

The weight value of biscuits was decrease gradually from 7.62 to 7.07g with increasing proportion of pumpkin flour. The highest value was of sample A (7.62g) and the lowest was found in sample F (7.07g). The results differed significantly among samples ($p<0.05$). As can be seen, the higher-level pumpkin flour incorporated, the more the weight loss of the biscuits. From the results above, the water absorption capacity of pumpkin flour (332%) had higher than the wheat flour (172%), hence, this resulted in the higher initial moisture content of the dough and the higher loss of water during baking of the biscuits [39]. However, the differences of two attributes among samples were insignificant ($p<0.05$). The densities of biscuits ranged from 0.83 to 0.98 g/cm3, with the highest value was of sample F (100% of pumpkin) and the lowest value was of sample A (100% of wheat flour). Density index was recorded as best index of sensory texture of biscuits. Lower in density means better crispiness and higher textural value (Dogan, 2006) [38]. These results were similar with the observation of Sneha *et al*. on biscuits from quality protein maize supplemented wheat flour.

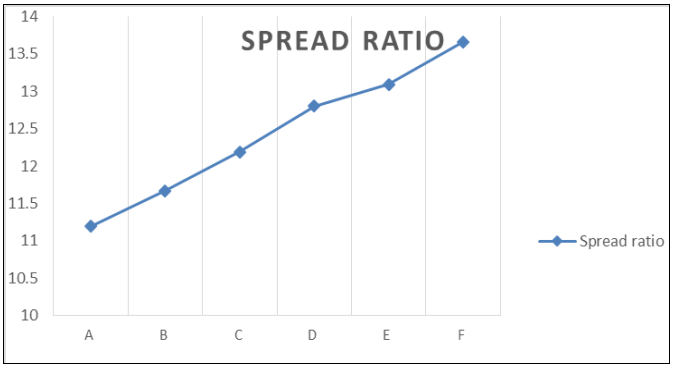

Fig 4: Effect of incorporating different levels of pumpkin flour on the spread ratio of biscuits.

Proximate values of developed biscuits

From the Table 5, the effect of the incorporation of pumpkin flour to the physico-chemical composition of developed biscuits were clearly shown. The collected data revealed that the moisture content of biscuits reduced from sample A to sample F (from 5.34% to 4.56%). There were significant differences in moisture between the control sample A and other treatments when more than 40% pumpkin flour incorporated in the biscuit production ($p<0.05$), though sample C and D were significant difference.

Table 5: Proximate values of developed biscuits

Sample	Moisture (%)	Fat (%)	Ash (%)	Fiber (%)	Protein (%)	Carbohydrate (%)
A	5.34 ± 0.04^{a}	13.70 ± 0.08^{a}	1.89 ± 0.03^{c}	0.25 ± 0.00	8.56 ± 0.08^{d}	70.89 ± 1.24^{a}
B	5.31 ± 0.01^{a}	13.59 ± 0.12^{a}	2.21 ± 0.42^{bc}	0.95 ± 0.00	8.73 ± 0.12^{cd}	69.39 ± 1.26^{ab}
C	5.04 ± 0.01^{b}	13.49 ± 0.14^{a}	2.56 ± 0.26^{ab}	1.85 ± 0.00	9.09 ± 0.04^{bc}	67.94 ± 0.05^{bc}
D	4.99 ± 0.04^{b}	13.41 ± 0.09^{a}	2.67 ± 0.04^{ab}	2.06 ± 0.00	9.28 ± 0.29^{ab}	67.62 ± 0.51^{bc}
E	4.67 ± 0.03^{c}	12.70 ± 1.23^{a}	2.91 ± 0.05^{a}	1.61 ± 0.00	9.46 ± 0.14^{ab}	68.47 ± 0.66^{bc}
F	4.56 ± 0.04^{d}	11.91 ± 1.03^{a}	3.05 ± 0.19^{a}	3.34 ± 0.00	9.67 ± 0.07^{a}	66.84 ± 0.03^{c}

*Values in the table represent the means standard deviations (n = 3 replicates).
The values denoted by different letters in the same column are significantly different (p ≤0.05)

The levels of fat in the biscuits ranged between 11.91% and 13.7% and the highest concentration of fat was found in biscuits A. Though there was a slight change in the fat content in different types of biscuits, it was statistically similar (p>0.05). Based on collected data, fat content decreased as the amount of pumpkin flour increased. This was probably due to the lower fat retention ability of pumpkin flour comparing with that of wheat flour during baking process. The mouth feelings and retaining the flavor of the biscuits improved when increasing the fat retention. The finding agrees with Jesmin *et al*. (2016) [41] on their reports for the decreasing trend in the fat content from pumpkin powder.

Based on figure 3, it is clear that the ash content of biscuits was improved when there was increased level of pumpkin flour. The clear effect could be seen when more than 40% pumpkin flour was used in making biscuits. Hence, the biscuits sample (F) had the highest ash content (3.05%), while the reference sample (A) had the least value (1.89%). There were significantly higher from sample C to sample F (p< 0.05) comparing with that of the control sample A (1.89%). These results had same increasing trend of ash content with those reported by (Giami, Achinewhu, & Ibaakee, 2005) [42]. The ash content of food material could be known as an indication of mineral constituents of the food because of that it is the inorganic residue remaining after the water and organic matter have been removed using heat with the presence of an oxidizing agent (Onabanjo & Dickson, 2014) [15].

The crude fiber content of biscuits produced from wheat-pumpkin composite flour also increased inhomogeneous significantly (p< 0.05) as pumpkin flour was supplementary to wheat flour in biscuits making. The results were obtained in range from 0.25 to 3.34%. The maximum value of crude fiber was created in sample F (3.34%) having 100% pumpkin flour substitution and the lowest were seen in sample A (0.25%) having 100% wheat flour. This led to pumpkin flour had more crude fiber than wheat flour. Biscuits E had 80% of pumpkin flour, which was recorded less fiber content (1.61%) than sample C (1.85%) and D (2.06%). This was due to different ratios between pumpkin flour and wheat flour. The result was in agreement with the observation of Giwa E & Abiodun V (2010) [43]. People who consume generous amounts of dietary fiber have health protective effect in comparison with those who have minimal fiber intake. Growing the amount of food, which contain high level of fiber can be protective against numerous health disorders such as diabetes mellitus, cardiovascular diseases, constipation, appendicitis, hemorrhoids, and colon cancer [44, 45] Fiber has characteristic as a buffering the pH of stomach by binding to the excess acids produced by the digestive system, aid in fecal bulking, and also intestinal emptying (Vergara-Valencia and others 2006). In addition, Following Dreher and Sharma (1981) [46] who reported that fiber components can get better texture, thickening, stabilizing, gelling and emulsifying. To sum up, pumpkin in baked product had health benefits with rising fiber content and other functional purposes.

Protein content increased with increase of substitution of pumpkin flour. The results of protein content in biscuits were observed in range from 8.56 to 9.67%. The biscuits sample (F) had the high percentage of protein content (9.67%), at the same time as the biscuits sample (A) had the lowest (8.56%). It was found that protein content for pumpkin-wheat biscuits was significantly higher (P ≤ 0.05) as compared to wheat flour. During growth, pregnancy and when recovering from wounds, protein is played extremely important that is a necessary constituent of all body tissues, which the new tissues is produced in the body. Increasing in the proportion of pumpkin flour which at the same time the carbohydrate content also increased. According to the further test result, it showed that the carbohydrate content among treatment group was significantly different from the control group. The lowest carbohydrate content is sample F (66.84%) and the highest value was presented in sample A (70.89%).

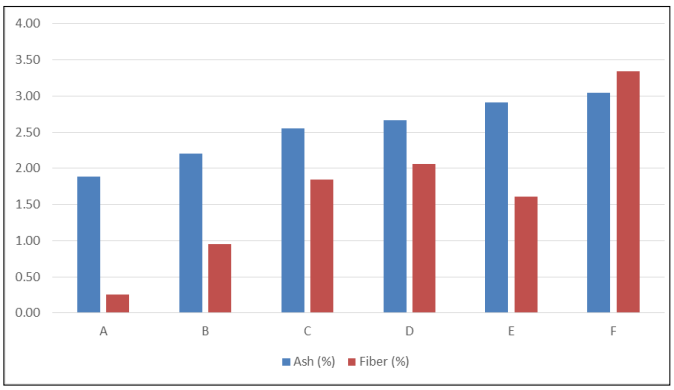

Fig 5: Effect of incorporating different ratio of pumpkin flour on the Ash and Fiber content of biscuits

Total Beta-Carotene Content of pumpkin flour and developed biscuits

Table 6: Total Beta- Carotene Content of pumpkin flour and developed biscuits

Sample	Beta – Carotene (mg/100g)
Pumpkin flour	28.8
Biscuits from pumpkin flour	4.02

As shown in the Table 6. The total β- Carotene found in pumpkin flour and biscuits from pumpkin flour. Beta-Carotene as natural colors instead of synthetic colors also increase vitamin A deficiency. β- Carotene is one of the plant carotenoids converted to vitamin A in the body. It works most efficiently in combination with other carotenoids and has the main purpose to reduce the risk of lung and colon cancer. Pumpkin also protect against certain cancers and cataract and is a powerful ally against degeneration aspect of aging. According to (Danilchenko *et al* 2003) [47], pumpkin fruits contain higher amount of β-carotene (0.4 mg/100 g). The results of total β-carotene of pumpkin flour in this study were recorded as high value (28.8mg/ 100g). When baking the pumpkin flour to make the biscuits, the total β-carotene was decreased from 28.8 to 4.02mg/ 100g. This led to β-carotene was be changed during baking process. This is higher results than the observation by (Chandler & Schwartz, 1988), which claimed that the carotene content was decreased up to 31.4% during baking. However, after baking, biscuits from pumpkin also retains amount of β-carotene. Therefore, biscuits which make from different ratios of pumpkin flour were feasible

Sensory evaluation

The level of acceptance of the product is determined by the hedonic test, which is test that used commonly [46,47]. Category-type scale which has odd number from 5 to 9 is the scale above, from "dislike" to "like extremely". The panelists included in the test should be consumer. This is a necessary step for developing and introducing the product, and no researcher can know if the product will be accepted or not [48]. There out, opinion of individual consumers can be projected to suggest directions for product optimization [48].

The produced biscuits using pumpkin flour plus with different ratios of wheat flour were sensory evaluated and compared with control biscuits with 100% wheat flour (sample A). The results five sensory assessment (color, taste, flavor, texture, overall acceptance) of biscuits from wheat- pumpkin flour are presented in Table 8. It is clear that the percentage of biscuits containing 40% of pumpkin flour (sample C) is the most acceptable. At 40% amount of pumpkin flour, all five attributes obtain the highest scores. Kulkarni and Joshi (2013) [51] reported that for the biscuits production, the replacement of wheat flour by pumpkin powder led in the direction of increase the color 274. The color of biscuits made from wheat-pumpkin composite flour was preferable than that of the control sample, which recorded in range from 2.9 to 3.55. The highest score was the sample C (3.55) containing 40% of pumpkin flour, while the score of control sample was the lowest, namely 2.9. In terms of taste, sample C was the most preferred with a score of 3.57, followed by sample E containing 80% pumpkin flour, namely 3.50. Similarly, in the terms of taste, the flavor scores of sample C was also recorded highest value (3.83) and the least score was of control sample (sample A). There were significant differences in texture ($p<0.05$) and the results of texture evaluation had ranged from 3.12 to 3.75. The results of the evaluation also showed that biscuits made from the composite flours of wheat and pumpkin were more accepted than the control ones. The highest score was of sample C (3.80), followed by sample D with the score 3.68 and the last place was sample A with the score as 3.12.

Table 7: Sensory evaluation scores of developed biscuits in term of color, taste, flavor, texture and overall acceptability in 5 - point scale.

Sample	Color	Taste	Flavor	Texture	Overall acceptance
A	2.90^{b}	3.04^{c}	3.18^{c}	3.12^{b}	3.12^{c}
B	3.28^{ab}	3.43^{abc}	3.46^{bc}	3.32^{ab}	3.28^{bc}
C	3.55^{a}	3.57^{a}	3.83^{a}	3.75^{a}	3.80^{a}
D	3.16^{ab}	3.34^{abc}	3.56^{ab}	3.40^{ab}	3.68^{ab}
E	3.22^{ab}	3.50^{ab}	3.36^{bc}	3.18^{b}	3.34^{abc}
F	3.28^{ab}	3.06^{bc}	3.36^{bc}	3.28^{ab}	3.22^{bc}

*Average of 50 evaluations. The values denoted by different letters in the same column are significantly different ($p \leq 0.05$)

Conclusions

- In this study, the replacement potential of the wheat flour by pumpkin flour in biscuits to improve nutritional values and the development of new recipes to make good quality biscuits from the pumpkin were successfully and thoroughly investigated.
- The chemical analysis of the pumpkin-wheat biscuits revealed that the fiber, ash and protein of the samples were significantly increased with at least 40% pumpkin flour by the selected substitutions. According to the sensory attributes scores, the results of biscuits produced from wheat- pumpkin flour composites were significantly higher than biscuits produced from 100% wheat biscuits in terms of all sensory attributes. The results also showed that the biscuits exhibited that the greatest acceptable sensory characteristics among consumer panel members were those containing 40%, followed by 60% pumpkin flour.
- In conclusion, the obtained outcome of this study stated that replacement wheat flour by different ratios of pumpkin flour to developed biscuits were not only improved in nutritional value and health benefits, but also highly accepted by various age of consumers.

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