Journal of Food Stability

ORIGINAL RESEARCH

Formulation and production of Spiced Pepper (*Capsicum chinenses* **Linn.**) Cube

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Abstract

Pepper is a high perishables and seasonal fruit. The aim of this work was to reduce the post-harvest losses of *Capsicum chinenses* Linn. by diverting the preservation techniques and valorise the product. A survey was carried out in Ngaoundéré, Adamawa region, Cameroon to know the level of pepper consumption, and the ingredients commonly added in pepper sauces. A simplex lattice mixture design was used to optimise the pepper cube formulation, which was therefore characterized. The sensory evaluation was done to select the best sample among those produced. From the survey, 76.92% of the population consume pepper. Onion, garlic, white pepper, and *pebe* were found to be the spices commonly used along with pepper sauces preparation. The pepper cubes were obtained by incorporating onion and *pebe* at different proportions. The enriched pepper was formulated at the optimal condition of the basic ingredients which were 0.817, 0.133 and 0.05% respectively for pepper, white pepper and garlic. Sample 107 with the colours ranging between orange yellow and yellow, made up of 50% pepper, 32.5% onion and 17.5% pebe was selected through sensorial analysis by the panellist as the best specimen. This product exhibited about 50% of 2,2-diphenyl-1-picryl hydrazyl (DPPH) scavenging activity.

Practical Applications

The pepper cube produced in this work is made up from local natural resources: pepper, garlic, onion, *pebe* and white pepper which contribute to the antioxidant capacity of the final product. Compared to the existing cube this can be used as natural antioxidant and does not content monosodium glutamate which is one of the main cause of hypertension.

Keywords: Pepper, spices, formulation, cube, antioxidant.

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1. Introduction

The increase in size and proportion of urban population raises the demand for food and hence the need for urban agriculture (Ludovic & Philippe, 2011). In order ro contribute to the nation's industrial and economic growth while maintaining quality and satisfying consumers' needs, pepper (*Capsicum chinenses*) has been used as local product of interest, as nowadays, consumers' interest for fresh or natural foods is significantly increasing (Djeukeu *et al.*, 2013). It is a perishable fruit which starts life green and turns red, orange, yellow or purple upon maturation (Vijaya & Sasikala, 2013). It is ranked the second valuable fruit after tomatoes with an estimated total production of 88,000 metric tons in 2011 (FAOSTAT, 2011). Capsicums are important food additives in many parts of the world, valued for their sensory attributes of colour, pungency and aroma (Estrada *et al.*, 2002). Consumers are becoming very selective on the products they purchase and hence demand a wide variety of products of high quality and good nutritional value (Nielsen, 2003). As a result, they are more concerned about what they eat and the impact on their health, reason why they take the information found on the products labels seriously and their choices highly depend on that. Consequently, industries have now been involved in the formulation and production of foods obtained by associating or mixing diverse raw materials from either natural or synthetic origin, this in order to produce foods that meet the consumers' and market demands (Nielsen, 2003; Jean-Marie & Gilbert, 2006). Formulation in food industries as reported by Jean-Marie & Gilbert (2006) is mostly done using ingredients from natural origin in order

to increase the performance of a product while enhancing its conservation and utilization. In the same line, it is necessary to give an added value to some local ingredients (garlic, onion, *pebe* and white pepper) through the formulation of a pepper (*Capsicum chinenses*) cubes in order to improve its performance and flavouring attributes.

Due to carotenoids, ascorbic acid, tocopherol, and other phytochemicals content (Kidmose *et al.*, 2006; Paksoy & Uslu, 2006), pepper consumption can reduce the risk of diseases, such as arthritis, cardiovascular disease, cancer and also delay the aging process (Packer, 1996). The major preservation forms of this fruit are pepper powder, pepper oleoresin (pepper oil) and preservation in acid brine (Tchiégang & Ngassoum, 1999; Tchiégang *et al.*, 1999; Naima *et al.*, 2013). To the best of our knowledge, pepper preservation in the form of cube has not yet been done. The objective of this work was to diversify the preservation technique and valorise pepper. Specifically, the work consisted in formulating fresh pepper cube with white pepper, garlic, onion and *pebe*, followed by evaluating its nutritional and sensory properties and finally determines the antioxidant properties of the final product.

2. Material and methods

2.1. Material

The biological materials used for this study were pepper, white pepper, garlic, onion and *pebe* (Figure 1).
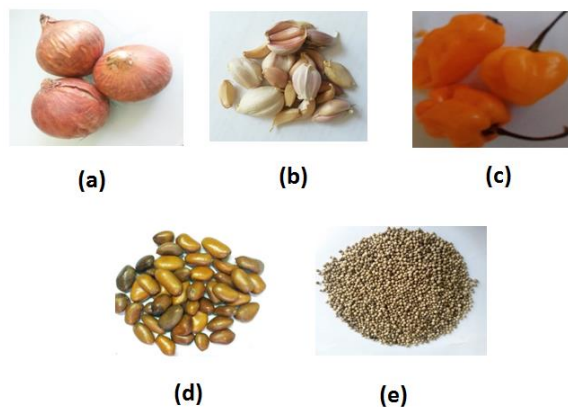
Figure 1: Plant materials used in this study: **(a)** = *Allium cepa* (Onion), **(b)** = *Allium savitum* (Garlic), **(c)** = *Capsicum chinenses* (Pepper), **(d)** = *Monodora myristica* (Pebe), **(e)** = *Piper nigrum* (White pepper)

Yellow pepper was harvested from a farmland at Boussirai, Ngaoundéré (Adamawa Region, Cameroon). *Pebe*, *White pepper*, Garlic and Onion were bought from a local market in the same city. *Pebe* and white pepper bought in the local market were coming from Yokadouma (East Region) and Penja (Littoral Region) respectively, while garlic and onion were coming from Garoua (North Region). Table 1 presents the plant material names, their family, the forms and parts used.

Table 1: Names, family forms and part of the biological materials used

Scientific name	Common name	Family	Forms and Parts used
Capsicum chinenses L.	Pepper	Solanaceae	Dried fruits
Monodora myristica G.	*Pebe*	Annonacea	Dried kernels
Piper nigrum L.	White pepper	Piperaceae	Dried berries
Allium sativum L.	Garlic	Liliaceae	Fresh bulbs
Allium cepa L.	Onion	Liliaceae	Fresh bulbs

2.2. Methods

2.2.1. Survey on pepper consumption

The investigation of pepper consumption was carried out with women who are the main marketers, processors, buyers, and users (Yaméogo *et al.*, 2002). During this survey, information concerning the preservation methods, the spices used as well as their appreciation of the new forms of pepper preservation in the town of Ngaoundéré-Cameroon were collected. Data were randomly collected from sixty five (65) selected women. This investigation was carried out with the aim of knowing the types of spices commonly used by women, as well as having an idea of the minor and major proportions used, so that it will facilitate the experimental design. A questionnaire was given to participants and the responses registered.

2.2.2. Scheme of work

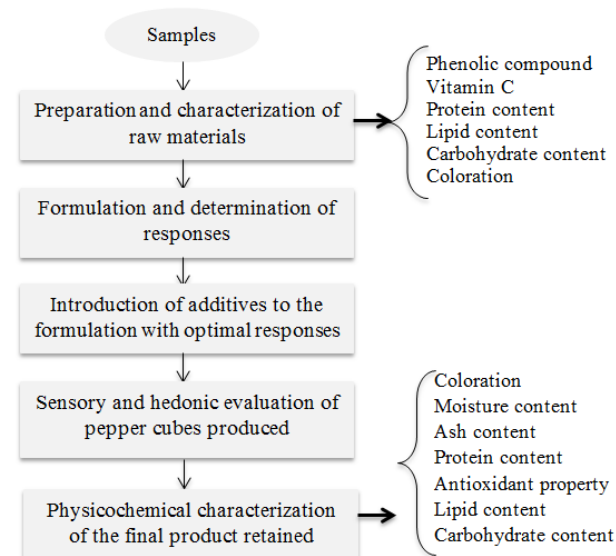A sequential advancement represented in figure 2 was used to achieve the objectives of this study.

Figure 2: Synoptic diagram of the work done

2.2.3. Samples Preparation

The studied samples were prepared according to their nature and base on the unit operations which are briefly explained below:

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Sorting: The raw materials (fresh yellow peppers, white pepper, *pebe*, garlic and onion) were selected manually. At this step, immature fruits/bulbs/grains, insects, bad or rotten fruits/bulds/grains, debris, stones and other impurities were eliminated.

Washing: The peduncles of the good fruits of the yellow pepper were removed and the fruits washed and rinsed with distilled water. Also, good garlic and onion bulbs were washed using distilled water to avoid product contamination.

Drying: The clean pepper fruits were dried at $55^{o}C$ in a ventilated oven for 7 days. They were then dried in order to reduce discolouration and oxidation of carotenoids as well as the oxidation of fats, proteins and capsaicin found in the seeds. It has been shown that the color of crushed or ground chilli powder deteriorates faster after drying than the normal chilli, due to the auto-catalyzed degradation of carotenoids (Berke and Shieh, 2002).

Dehulling: *Pebe* nuts were dehulled manually and the shells removed.

Grinding: White pepper, *pebe*, garlic and onion and pepper itself were ground using a ZAIBA® blender with model number ZB-2255, China.

Sieving : The ground samples were then sieved using an Analysensieb 500 μm sieve, model number 305714 to obtain particle sizes of less than or equal to 500 μm.

Peeling: Garlic and onion were peeled and the peels discarded. They were ground immediately after washing and prior mixing, in order to prevent oxidation and discoloration.

Blending: White pepper, Garlic and pepper were mixed with the help of a blender to obtain a product that was preliminary tested. Onion and *pebe* were further added to the formula and mixed thoroughly to obtain a spiced product.

Moulding: The spiced product obtained after was then shaped and preserved in the refrigerator to increase its shelf life.

2.2.4. Formulation of pepper cubes

The formulation of pepper cubes using five required the mixture of ingredients in appropriate proportions so as to obtain a homogeneous product equivalent to 100 %. Figures 3 and 4 clearly outline the unit operations used in this process. Each constituent had a percentage that depended on the others as described by Goupy & Creighton (2006). According to these authors, when the concentration of one of the ingredients increases that of the other reduces.

A simplex lattrice mixture design was carry out to optimise the pepper cube formulation. The experimental domain of the major component (pepper) was set as 70-85 % while white pepper and garlic had as domains 10-25 % and 5-20 % respectively. All parameters were coded as 0 (zero) for the lowest limit and 1 (one) for upper limit. Minitab 14 was used as software to generate the experimental matrix for the formulation and analysis of experimental data. Table 2 clearly shows the experimental matrix where X_1 represents
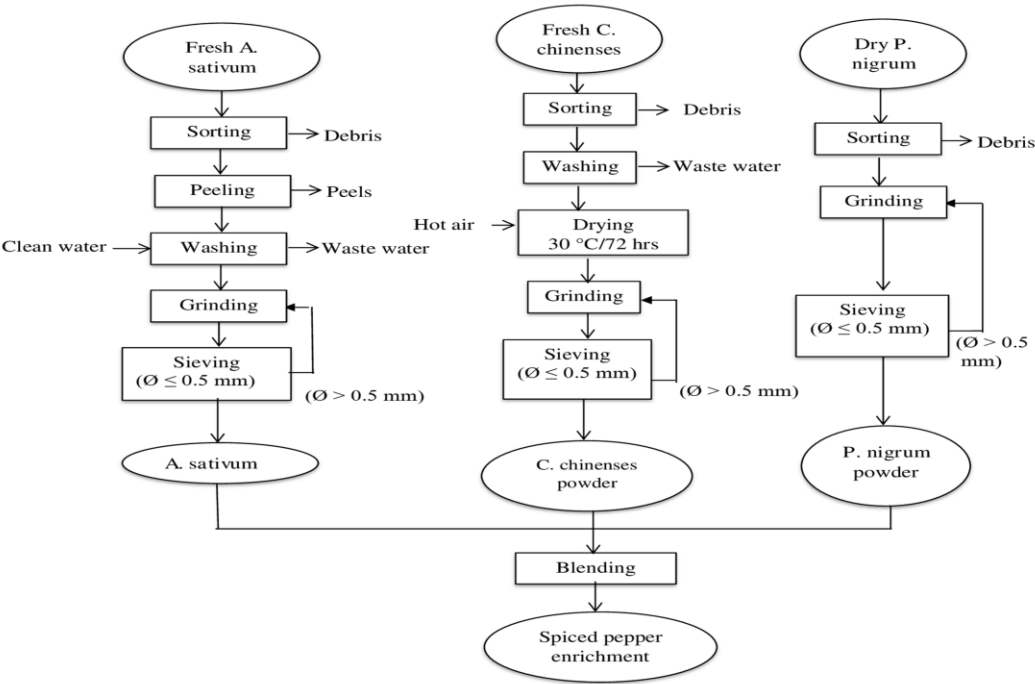
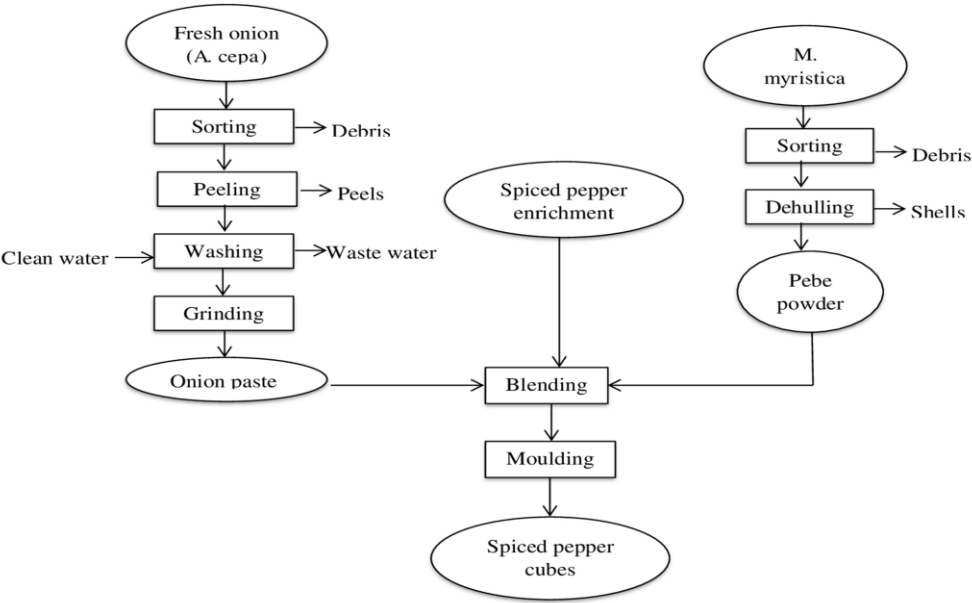**Figure 3 :** Production process of spiced pepper paste

Figure 4 : Production process of spiced pepper cubes

pepper, X_2 White pepper and X_3 garlic proportions, while table 3 indicates the same parameters in reel values. Ten experiments were carried out in order to define the optimum proportions of the basic components (pepper, garlic and white pepper) using vitamin C content as response. Vitamin C content was chosen as response because it is an antioxidant component and stands as an indicator for antioxidant activity for the final product. It is also very unstable and capable to undergo transformations if exposed to heat, light and oxygen.

Table 2: Varying proportions of pepper, white pepper and garlic as pseudo-components

Trials	X1	X2	X3
1	0.000	1.000	0.000
2	0.167	0.667	0.167
3	0.000	0.000	1.000
4	0.667	0.167	0.167
5	0.500	0.500	0.000
6	0.000	0.500	0.500
7	0.500	0.000	0.500
8	0.333	0.333	0.333
9	0.167	0.167	0.667
10	1.000	0.000	0.000

2.2.5. Enrichment and production of spiced pepper

The desired cube product should be rich vitamin C, phenolic compound so that its antioxidant activity will be high. In view of obtaining a product with the above mentioned expected characteristics, onion and *pebe* were used as additives in different proportions.

According to Vaclavik & Christian (2008) food additives are substances or mixture of substances other than a basic foodstuff, added to foods for technological purpose. Onion was used in high amount compared to *pebe,* because of its technological role i.e. its high moisture content was used to humidify the powders so as to facilitate moulding upon compression. *Pebe* was chosen and used to improve the aroma of the pepper cube since the kernels possess a very strong aroma as reported by Enwereuzoh *et al.* (2015). The desired mass for a cube was 10 g and table 4 presents the different proportions of ingredients introduced as additives in 50 % pepper cube. Therefore, as shown in table 4, the additives weight varied from 3 to 4 g for onion, 1-2 g for *pebe* and the mass of the enriched pepper maintained at 5 g.

Table 3: Varying masses (g) of pepper, white pepper and garlic during formulation

Trials	X1	X2	X3	Vitamin C
1	0.700	0.250	0.050	Vit C_1
2	0.725	0.200	0.075	Vit C_2
3	0.700	0.100	0.200	Vit C_3
4	0.800	0.125	0.075	Vit C_4
5	0.775	0.175	0.050	Vit C_5
6	0.700	0.175	0.125	Vit C_6
7	0.775	0.100	0.125	Vit C_7
8	0.750	0.150	0.010	Vit C_8
9	0.725	0.125	0.150	Vit C_9
10	0.850	0.100	0.050	Vit C_{10}

Table 4: Varying masses (g) of onion and *pebe* in different formulations

Range	Pepper enrichment	Onion	*Pebe*
Sample A	5.00	3.00	2.00
Sample B	5.00	3.25	1.75
Sample C	5.00	3.50	1.5
Sample D	5.00	3.75	1.25
Sample E	5.00	4.00	1.00

2.3. Physicochemical characterization of the samples

2.3.1. Chemical analyses

2.3.1.1. Dry matter and ash content

The determination of the dry matter of samples was done using the AFNOR (1982) method and the ash content was performed using the method described by AOAC (2002).

2.3.1.2. Total proteins, lipids and carbohydrate content

The total nitrogen was determined after mineralization of the samples according to the Kjeldahl method, using boric acid solution in the presence of an indicator as described by AACC (1999). The nitrogen value was then converted into total protein content by multiplying with 6.25 as conversion factor. The total lipids were extracted using the Soxhlet extractor as described in the Russian method described by Bourely (1982). The total nitrogen was then converted into total protein content by multiplying with 6.25 as conversion factor. The total carbohydrates were calculated as described by FAO (2008), using the following formula:

Total Carbohydrate contents (%) = 100 − (% [protein + fat + moisture content])

2.3.1.3. Determination of Vitamin C and total phenolic contents

The vitamin C content was determined by the official AOAC titrimetric method using the 2, 6 Dichlorophenol indophenol (AOAC, 1990). While the crude total phenolic compounds were extracted with 70% ethanol and then assessed with the Folin - Ciocalteu reagent as described by (Marigo, 1973).

2.3.1.4. DPPH radical scavenging activity assay

The antioxidant activity was determined by the capacity of a component to scavenge a free radical or to give an atom of hydrogen according to the method described by Zhang & Hamauzu (2004), with slight modifications. 2 ml of freshly prepared DPPH solution (0.1 mM prepared in methanol) was introduced into a test tube containing 0.5 ml of extract sample. The mixture was thoroughly stirred for 5 min and incubated in the dark for 30 min at ambient temperature (25°C). For the control tube, methanol was used to replace the extract. The absorbance of the resulting solution was red at 517 nm, and the antioxidant activity of the extract calculated as follow:

$$\textbf{Scavenging Activity effect} (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample).

2.3.2. Physical analyses: Color

The color of the cubes was determined using the CIE L, a, b coordinates L*, a* and b*, where L*, a* and b* values were recorded,

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with L* indicating lightness (+) or darkness (-), a* indicating red (+) or green (-) and b* indicating yellow (+) or blue (-). The three values are needed to completely describe the color of an object (Ismail *et al.*, 2015). The samples were placed on a clean white paper and introduced in a wooden box for image acquisition. The image was introduced in the computer for pre-processing, segmentation and color conversion from RGB to Lab space, using the ''Image J'' software. The measurements were made directly on the upper surface, which was always steady, of cylindrical samples and the L*, a* and b* values were recorded. The color intensity (C) and the hue angle (h_{ab}) were calculated using the formula below:

$$C = \sqrt{a*^2 + b*^2}$$

$$h_{ab} = \tan^{-1}(b*/a*)$$

2.4. Sensory analysis and hedonic evaluation

A descriptive sensory analysis was the method chosen, because its helps to discriminate a wide range of products based on their sensory characteristics and it also helps panelists to identify and quantify a product's sensory property Singh-Ackbarali & Maharaj (2014). Hedonic evaluation was also done according to the method described by Aka-Boigny *et al.* (2016), with some modifications to determine the degree of consumer acceptance or preference of the product. For this evaluation 12 panellists were randomly chosen based on their frequency and their awareness in pepper consumption and use. They were informed on the objectives of the analysis and were taught on how to proceed. Five pepper cube samples with different proportions of additives and different codes, were presented to each panelist

with potable water. Bread was provided to them also for chewing and rinsing of the mouth after each sample. A 9- point scale form ranging from (9)-like extremely to (1) disliked extremely, was given to each panelist to score the following parameters: appearance (color), shape, size, flavor, friability, and the overall acceptability of the product.

The radar plot was used to represent the panelists' view of each sensorial characteristic tested.

3. Results and discussion

3.1 Survey on pepper consumption

The survey was carried out in Adamawa region of Cameroon. Sixty five women were sampled with the aim to know the level of pepper consumption, the color of pepper used and the spices used during preparation. Out of the sampled population, it was essential to know the consumption rate of pepper and figure 5 shows the consumption level of pepper. From the figure, 76.92 % of the sampled population consume pepper and 23.08 % do not. Out of this percentage, 70 % use pepper directly in food during cooking while 30 % consume as supplement. Similar results were obtained by Tchiégang *et al.* (1999) on the utilization of pepper in human nutrition in Cameroon. These choices of pepper consumption can be explained by the fact that cooking directly in food saves time and gives an additional flavour to food.

Out of the individuals who consume pepper, 85.71% of them were found to consume pepper in its fresh form and 11.43% use the powder form to cook. While 2.86% go for both the powder and the fresh forms. These forms of
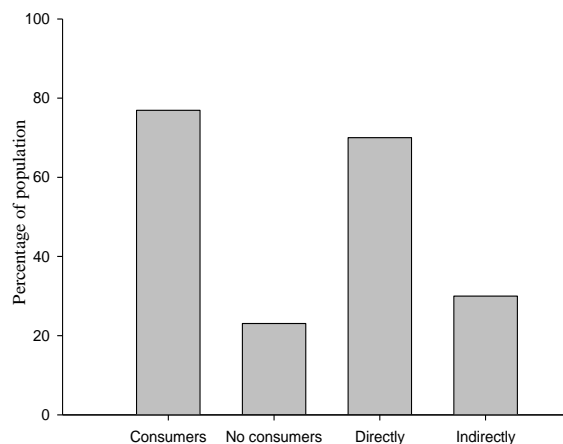
Figure 5: Representation of pepper consumers and non-consumers from the sampled population

Figure 6: Representation of consumers' pepper choice based on the color

pepper consumed are in agreement with the report of Segnou *et al.* (2013), who stated that pepper fruits are consumed fresh or dried either as whole fruit or ground. The preference for the fresh form is due to its availability, accessibility and reduced pungency as stipulated by Ozgur *et al.* (2011). The authors show that dried foods are more concentrated than fresh foods.

Figure 6 show that 50% of the sampled population prefers the yellow color, 30% the red color and 20% no particular preference. The yellow color is consumed more than the orange and red colors. This might be because the population desires pepper with mild pungency and orange peppers are not popular in Cameroon. In fact Tchiégang & Ngassoum (1999) reported that yellow peppers are less pungent than red peppers.

In order to know which ingredients are used along with pepper during pepper sauce preparation, the sampled population was interrogated. Figure 7 shows the ingredients commonly used by the women during the preparation of their pepper sauces.
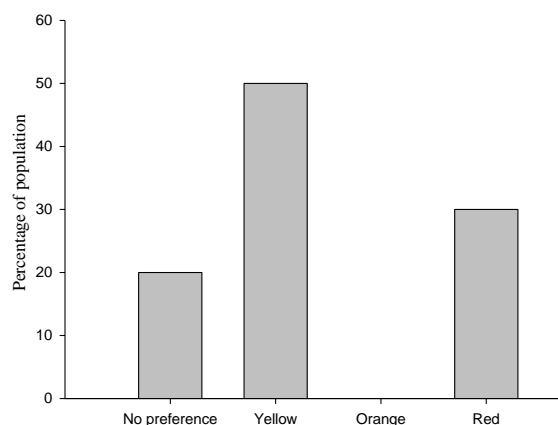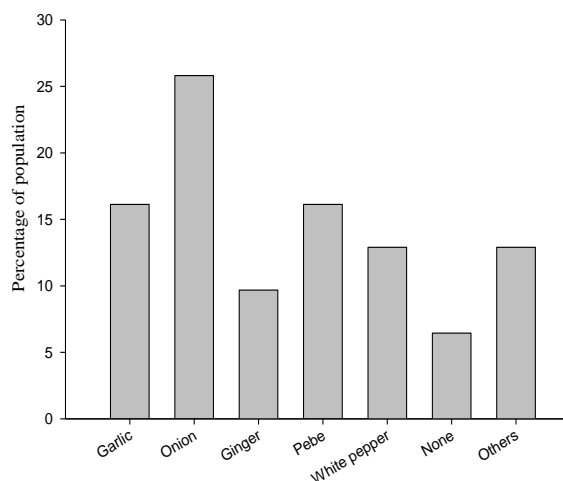

Figure 7: Representation of the ingredients used during the preparation of pepper sauce

3.2. Chemical properties of raw materials

The results obtained after analysis of the five raw materials (pepper, white pepper, *pebe*, garlic and onion) used during the production of pepper cubes are presented in table 5.

3.2.1 Vitamin C content

The vitamin C content of pepper was 12.88 mg/100 g which fall in the range 12.0 to 44.4 mg/100 g (Howard *et al.*, 1994) for red varieties. However, its vitamin C content was

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Table 5: Chemical composition of raw materials (% of fresh weigh)

Composition	Ingredients				
	Pepper	White pepper	*Pebe*	Garlic	Onion
Moisture content (%)	17.34±0.37	17.60±1.00	11.66±0.32	76.06±0.32	92.16±0.72
Vitamin C (mg)	12.88±1.98	6.52±0.77	28.54±1.06	1.07±0.45	1.75±0.41
Phenolic compounds (mg)	12.76±1.50	4.42±0.47	5.36±0.63	1.37±0.46	1.46±0.14
Lipids (%)	4.15±0.04	5.23±0.35	9.60±0.02	0.42±0.05	3.26±0.04
Proteins N x 6.25 (%)	19.90±0.71	15.75±1.93	24.45±4.71	18.73±0.56	3.62±0.04
Carbohydrate (%)	58.61±0.52	61.40 ±0.47	54.29 ±0.97	4.79 ±0.18	0.96 ±0.16

lower than that dried chilli which is about 26 mg/100 g (Kim *et al.*, 2006).

This difference can be due to the sensitivity of ascorbic acid to oxidation upon drying and exposure to light during grinding as well as to the maturity, growth conditions and cultivar. It has been reported that factors such as genotype, environment and fruit maturity affect the ascorbic acid content (Campos *et al.*, 2013).

3.2.2. Phenolic content

Among the ingredients used for pepper cube production, pepper has the highest phenolic content 12.76 mg/100 g, followed by *pebe* 5.36 mg/100 g and white pepper with a value of 4.42 mg/100 g. Onion and garlic presented the least values of 1.46 mg/100 g and 1.37 mg/100 g respectively. The phenolic content of garlic was 1.37 mg/100 g which is similar to the value obtained by Womeni *et al.* (2013) in Cameroon. *Pebe* had a value 5.36 mg/100 g which is fairly above 3.22 mg/100 g obtained by Womeni *et al.* (2013). This difference can

be attributed to the storage conditions of the spice.

3.2.3. Lipid content

The highest lipid content was recorded with *pebe* (9.60%). This value is close to 8.92 % obtained by Chibuzor & Assumpta (2014) with the same spice. This difference could be attributed to the difference in origin and variety of the products as well as the analysis methods used. White pepper and pepper showed fairly higher values of 5.23 % and 4.15 % respectively. High lipid content is indicative of the fact that these spices are good sources of flavor since they are rich in essential oil (Enwereuzoh *et al.*, 2015).

3.2.4. Protein content

The total protein contents of raw materials were determined using Kjeldhal method. From the five ingredients used the highest protein content was obtained with *pebe* (24.45%), followed by pepper (19.90%), garlic (18.73%) and white pepper (15.75%) while the lowest value for this biochemical parameter was

Table 6: Colour characteristics of the ingredients used

Samples	Colour parameters				
	L*	**a***	**b***	**C**	**H**
Pepper	65.27±2.45	3.04±1.28	56.67±1.28	56.76±1.34	86.94±1.23
White pepper	99.82±0.01	-5.76±2.71	17.31±9.01	18.25±9.39	108.92±1.90
Garlic	97.18±4.77	-10.66±0.70	37.28±3.0	38.77±3.07	105.97±0.29
Pebe	41.99±0.72	5.41±1.22	28.20±0.85	28.73±1.04	79.18±2.14
Onion	85.35±2.31	-8.29±0.76	19.32±0.88	21.03±0.98	113.20±1.69

L = lightness. a* = redness. b* = yellowness. C = color intensity. H = Hue angle (*in degree*)

recorded in onion (3.62%). The protein content of *pebe* (24.45%) is close to 27.57 % obtained by Enwereuzoh *et al.* (2015). This difference can be attributed to the stage of maturity of the spice at the time of harvest, the variety of cultivar and partly to the method of analyses used by Enwereuzoh *et al.* (2015).

3.2.5 Carbohydrate content

The total carbohydrate content of raw materials was calculated by difference. The highest value was recorded with white pepper (61.40%), followed by pepper (58.61%) and finally *pebe* (54.29%). The lowest carbohydrate content was found in onion (0.96%). The carbohydrate content obtained in this study was 54.29% which is higher than 44.84% obtained by Chibuzor & Assumpta (2014) with the same spice from Nigeria. This difference can be as the result of the origins and varieties of the samples.

3.3 Color

The physical parameter determined was color of the raw materials, Chroma and Hue values. From table 6, white pepper had the highest L*

value (99.82) followed by garlic (97.18) and onion (85.35). This could be due to the presence of starch which is whitish since studies carried by (Pruthi, 1980) showed that this spice contains 52% of starch. The white pepper was the most brilliant ingredient amongst the 5 spices while the least brilliant was *pebe* with an L* value of 41.99. The b* value of 56.67 for pepper was found to be the highest value, followed by garlic (37.28). These values revealed that they are both more yellowish in color than the rest of spices where white pepper was seen with the least value of 17.31. Meanwhile *pebe* with a value of 28.20 was observed to have more yellowish color than onion. The color variation in these spices can be attributed to varied quantities and types of color pigments found in them since the spices are of different varieties, origin and families.

Chroma (C) for pepper and garlic were respectively 56.76 and 38.77. The value obtained for pepper was above 50 implying that the color tends towards a pure yellowish color. However, the product does not have the pure color possibly because of

several types of carotenoids that are found in it and at different proportions.

The Hue angle ranged from 0 to 360°. The hue angles for white pepper, garlic and onion were 108.92, 105.97 and 113.20 respectively. From the Hue disc, these samples colors were lemon yellow and yellow green, showing that these spices content molecules that are responsible for slight green color registered. *Pebe* and pepper had Hue values of 86.94 and 79.18 respectively and from the Hue disc, these spices colors ranged between orange yellow and yellow. This can be attributed to the presence of color pigments.

3.4 Production of pepper cubes

The Simplex Lattrice Design was carried out through 10 experiments to determine the appropriate quantity of the principal ingredients used in pepper cubes formulation.

Table 7 presents the experimental matrix carried out along with different responses obtained for each trial. X1, X2 and X3 represent the proportions of pepper, white pepper and garlic respectively. Table 7 shows the results obtained considering vitamin C content as response for 10 formulations.

Formulations 1, 5 and 6 gave similar vitamin C content (6.963 mg/100 g) that was the highest response among all. The lowest (4.940 mg/100 mg) response was obtained from the third formulation.

To better understand the influence of different factors on the response, the contour plot was drawn (figure 8) using Minitab as software. This figure represents the evolution of the response surface according to the experimental domain of the 3 factors (pepper, white pepper and garlic).

Table 1: Experimental matrix and different responses for the mixture design

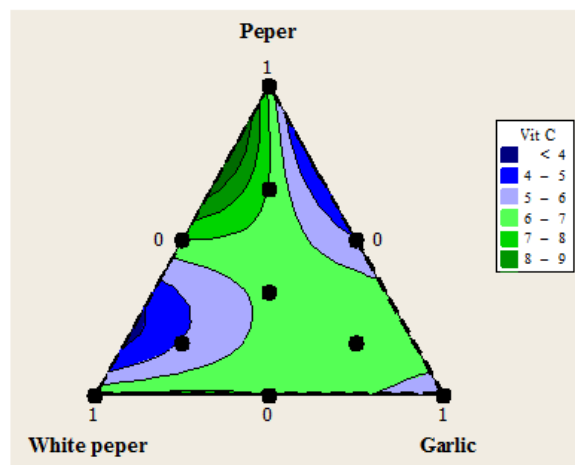| Trials	X1 (g)	X2 (g)	X3 (g)	Responses
1	0.700	0.250	0.050	6.963
2	0.725	0.200	0.075	5.056
3	0.700	0.100	0.200	4.940
4	0.800	0.125	0.075	6.900
5	0.775	0.175	0.050	6.963
6	0.700	0.175	0.125	6.963
7	0.775	0.100	0.125	5.065
8	0.755	0.150	0.100	6.259
9	0.725	0.125	0.150	6.690
10	0.850	0.100	0.050	6.591

Figure 8: Mixture contour plot for Vitamin C content

Table 8: Analysis of variance for vitamin C contents

Source	DF	Seq SS	Adj SS	Adj MS	F-value	P-value
Regression	8	6.79977	6.79977	0.84997	201.78	0.054
Linear	2	1.75328	2.31890	1.15945	275.25	0.043
Quadratic	3	0.79339	1.21296	0.40432	95.98	0.075
A*B	1	0.00097	0.02214	0.02214	5.26	0.262
A*C	1	0.22221	0.33248	0.33248	78.93	0.071
B*C	1	0.57020	0.67886	0.67886	161.16	0.050
Full Cubic	2	4.23558	4.13162	2.06581	490.41	0.032
A*B*(-)	1	2.76808	3.94175	3.94175	935.75	0.021
A*C*(-)	1	1.46750	1.23059	1.23059	292.14	0.037
Special Quartic	1	0.01753	0.01753	0.01753	4.16	0.290
A*A*B*C	1	0.01753	0.01753	0.01753	4.16	0.290
Residual Error	9	0.00421	0.00421	0.00421		
Total		6.80398				

DF: Degrees of Freedom and MS: Mean Square; SS: Sum of Squares; Adj-R^2 = 99.44%.
R^2=99.94%.

The regression model obtained from these responses is shown by the following equation:

Y= 6.59 A + 6.96 B + 4.94 C + 0.73 A*B - 2.82 A*C + 4.03 B*C +33.96 A*B* -18.98 A*C - 12.74 A*A*B*C

Where Y is the response, A (pepper), B (white pepper) and C (garlic), are the factor terms A*C, A*B, B*C and A*A*B*C are interaction terms.

The statistical analysis was performed at 5% probability level. Table 8 shows the ANOVA analysis of vitamin C content in the mixture design studied.

The linear and full cubic terms with p-values lower than 0.05; significantly influenced the vitamin C content of the product. In order to produce the pepper cube with high amount of vitamin C, the studied factors were optimized. Thus, the maximum amount of vitamin C content was obtained with A = 0.778, B = 0.222 and C = 0 as pseudo components values. The equivalent corresponding proportions (in %) of these components were 0.817, 0.133 and 0.05% respectively for A, B and C. The implementation of these optimal proportions gave 10.578 ± 0.619 mg as vitamin C content which was very close to the predicted value (10.056 mg). This result along with the higher value of the determination coefficient (R^2 = 99.94%) testified that the model proposed to

explain the phenomenon is acceptable and therefore can be used to predict the vitamin C content of the final product. In fact, to be acceptable, the coefficient of determination (R^2) should be higher than 80% (Joglekar & May, 1987).

3.5 Sensory evaluation and acceptability

The mean scores of the varied responses obtained from the hedonic and descriptive sensory tests of the five pepper cube samples are summarized in figures 9 and 10. Sample 107 had an overall preference over the other four followed by sample 218. This can be explained by the fact that sample 107 had the least pungency as a result of the smaller quantity of onion used. In fact onion has been reported to be pungent as a result of the interaction between S-substituted-L-cysteine sulfoxide derivatives and allinase enzymes (Pruthi, 1980). Hence 7.5 g of onion used in sample 218 contributed to the increment of the products pungency.
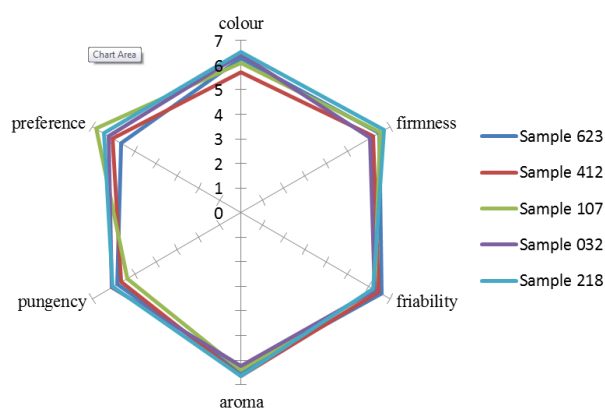

Figure 9: Hedonic and descriptive sensory tests of pepper cube samples

From figure 9, it was noticed that all samples fell within the acceptability zone with average mean scores ranging between 6 and 7. In order to select the best formulation among the five samples, samples 107 and 218 scores were drawn individually. Figure 9 shows the hedonic acceptance and descriptive sensory of the samples 107 and 218.
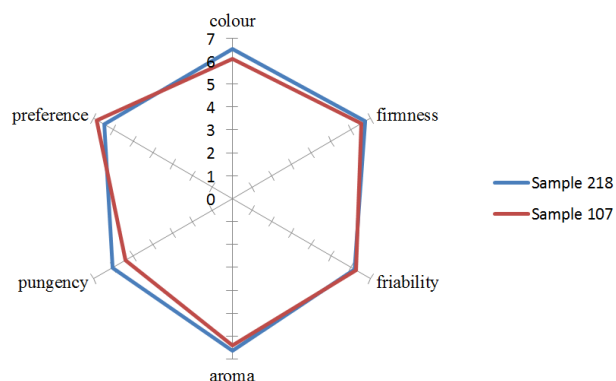

Figure 10: Hedonic and descriptive sensory tests of samples 107 and 218

From figure 10, it is clear that with the combination of the studied parameters, sample 107 was more preferred than sample 218. In fact, it its score was 6.83 and that of sample 218 6.47. Both samples were retained for further analyses.

3.6. Analyses of the retained samples

3.6.1. Chemical analyses

The retained samples were analyzed biochemically and results are represented in table 9.

The lipid content of sample 107 was 10.58% while that of sample 218 was 9.14%. The protein content of sample 107 (49.51%) was also slightly higher than that of sample 218 (47.24%). This difference can be due to the varied quantity of *pebe* used in their formulation. Sample 218 had

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Table 2: Physico-chemical properties of the retained samples (% of dry weight)

Parameters	Samples	
	Sample 107	Sample 218
Moisture content (%)	37.52 ± 0.13^a	43.36 ± 0.40^b
Ash content (%)	2.60 ± 0.00^a	2.86 ± 0.12^b
Protein N x 6.25 (%)	49.51 ± 0.08^a	47.24 ± 0.13^b
Lipid (%)	10.58 ± 0.02^a	9.14 ± 0.08^b
Carbohydrate (%)	0.28 ± 0.05^a	2.38 ± 0.09^b
Vitamin C (mg)	127.46 ± 2.14^a	110.44 ± 2.03^b
Phenolic compounds (mg)	69.28 ± 0.04^a	66.35 ± 0.46^b
L*	47.48 ± 5.01^a	35.40 ± 1.34^b
a*	0.94 ± 0.80^a	1.03 ± 0.43^a
b*	28.27 ± 3.67^a	24.39 ± 0.37^b
C	28.29 ± 3.68^a	24.42 ± 0.39^b
H	88.19 ± 1.58^a	87.60 ± 0.97^a

L* = lightness. a* = redness. b* = yellowness. C = color intensity. H = Hue angle (in degree)
The values in the same line with different superscripts letter are significantly different (p<0.05)

1g of *pebe* while sample 107 contained 1.75g and was the spice containing the highest lipid content among the five ingredients. The carbohydrate content of both samples were very low 0.28 and 2.38% respectively for sample 107 and sample 218. The ash content of sample 107 (2.60 %) was less than that of sample 218 (2.86 %) and this can be because of the solubility of some minerals in the available moisture provided by the onion. It can also be the result of the origins of the products' components since the chemical composition of plants are controlled by climate, soil, plant factors and species (Koyuncu *et al.*, 2014).

From these analyses, sample 107 was shown as having the highest vitamin C content (127.46 mg/100 g of dry weight) compared to sample 218 which exhibited 110.44 mg vitamin C/100 g of dry weight. Its phenolic content (69.28 mg/100 g) was also significantly higher than that of sample 218 (66.35 mg/100 g). This can be the result of interactions between the phenolic compounds and other constituents found in the other spices since phenolic compounds belong to different classes which react differently (Abdou *et al.*, 2010). The sample 107 can therefore be considered as having higher antioxidant property compared to sample 218 since ascorbic acid and phenolic compounds are good antioxidants (Maksimova *et al.*, 2014).

3.6.2. Color analyses

The L*, a*, b* Chroma and hue angle characteristics of the retained samples after sensory evaluation are summarized in table 5. The L* value (lightness) for sample 107 was

47.48 which is higher than that of sample 218 (35.40). Both samples had values below 50, showing that their colors are dark and the intensity of darkness higher in sample 218. The redness (a* value), were 0.94 and 1.03 respectively for samples 107 and 218, indicating that both samples are more green than red and this can be related to the presence of some specific molecules in garlic and onion since they exhibited the highest negative values (green) of -10.66 and -8.29 respectively. From the a* values, is clear that the green color in sample 218 was higher than that of sample 107. The yellow color of food products as specified by b* for both samples (107 and 218) were 28.27 and 24.39 respectively. The Chroma (C) or color strength describes the dullness of a color of the sample. It is the intensity and the saturation level of a particular hue (closest "pure" color). The Chroma values for samples 107 and 218 were 28.29 and 24.42 respectively. This indicates that the color intensity of sample 107 was close to the saturation level of pure pepper (56.76) compared to sample 218. This difference in saturation level between samples 107, 218 and pure pepper can be as a result of the low Chroma values of onion (21.03), white pepper (18.25) and p*ebe* (28.73). The Hue is the color attributed to an object. Quite simply, hue is how we perceive a sample's color red, orange, green, blue. It is generally ranged from 0 to 360°. White, black and gray have no Hue. The Hue angles for samples 107 and 218 were 88.19 and 87.60 respectively which falls within the desired range. From the Hue disc, these two samples eventually have colors found between orange yellow to yellow which can be attributed to the presence of pepper the main component in the products.

3.6.3. DPPH radical-scavenging activity

DPPH free radical scavenging method was used to evaluate the antioxidant activities of the retained formulations. Figure 11 shows that, both of the retained samples can scavenged the free radical at different proportions. In order to compare the DPPH radical scavenging activity of retained samples (107 and 218), ascorbic acid was used as reference and the IC_{50} values calculated (figure 12).
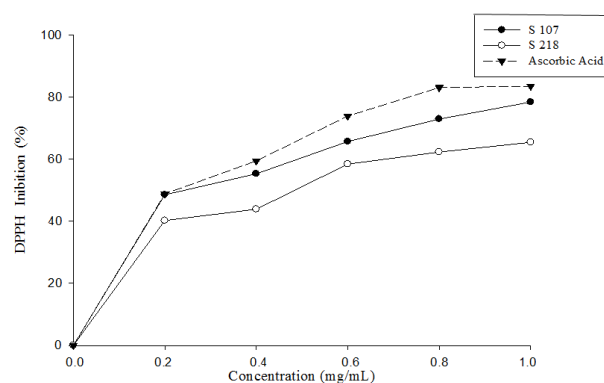

Figure 11: DPPH radical scavenging activity of the retained samples in comparison with ascorbic acid
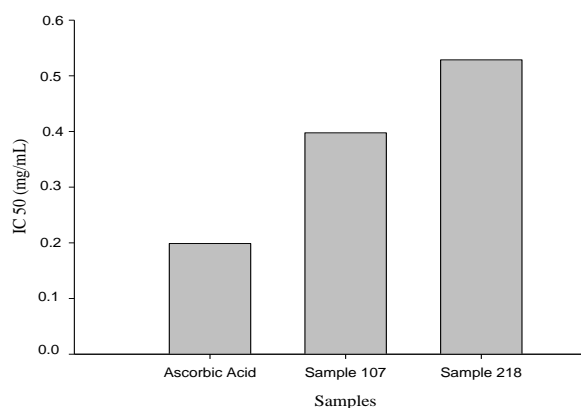

Figure 12: IC_{50} values of the retained samples and ascorbic acid.

The DPPH scavenging effect for sample 218 was lower than that of sample 107. This can

be due to the fact that, the vitamin C and phenolic contents in sample 218 were lower compared to that of sample 218. Both samples showed high IC_{50} values compared to ascorbic acid. However, sample 107 exhibited about 50% of DPPH scavenging activity. It was therefore selected as the most active sample.

Base on the above analyses, the best proportions for pepper cube making was defined to be: 50% of pepper, 32.5 % of onion and 17.5 % of *pebe*.

Figure 13: Spiced pepper cube

4. Conclusion

This work was carried out with the objective to formulate a pepper cube using white pepper, garlic, onion and *pebe* as ingredients. The sensory evaluation of the products showed that they can be accepted by consumers. This acceptance will help to improve its economic potential, and reduce cooking time. Compared to the existing cube these one are natural and do not content monosodium glutamate which is one of the main cause of hypertension.

Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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