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Original Research

Physico-Chemical, Surface and Thermal Properties of Date Palm Pollen as a Novel Nutritive Ingredient

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ABSTRACT

Background
Date palm pollen (DPP) is a natural product well-known in folk medicine in the Arab world. It is used to improve the fertility of human beings and studies have tested this activity on rabbits and rats. In the region of Sfax from Tunisia, a huge quantity of DPP could be discarded. Taking into account of the richness of this typical product of different components and of the trend of producing food supplements that could be sold at medium price comparing with the existing product, DPP was analyzed on the basis of physical and chemical properties in order to promote its use as a techno-functional ingredient in the agri-food and pharmaceutical field.

Results
X-ray diffraction showed that DPP is characterized by an amorphous structure which leads to better techno-functional properties while stored in a water-air-tight container. Findings proved that DPP is capable to reduce the surface tension. Collected data from thermal analysis proved that DPP is thermally stable during storage and in different food systems.

Conclusion
The present study demonstrated that DPP could be used in the agri-food and pharmaceutical field. The obtained results help to define the suitable storage conditions of DPP and to predict its behavior when used as an ingredient. DPP can be used as a whole in food formulations or after extracting protein which is the main responsible agent for surfactant property. DPP proteins might be used as a food supplement in commercial sports nutrition products that can be sold at medium prices compared to some existing products.

Keywords
Date palm pollen; Physico-chemical; Morphology; Surface; Thermal.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious and a wind-pollinated species. The fine powder used to pollinate date palm females is commonly known under the name of pollen. The latter is the male gametophyte used for the safe transportation of the genetic material. It is formed in the anther and germs by generating a tube that allows the ovule fertilization in female stigma.

Pollen is a natural source of biochemical and nutritional substances such as protein, carbohydrates, amino acids, minerals, sterols, hormones, and many different kinds of enzymes and co-factors.[1] Its composition may widely vary between species. For example, maize pollen is characterized with 23.80% protein, 13.92% oil, 22.04% total sugar,[2] while hardy kiwi pollen has 26.5% protein, 7% fat and 5.3% ash.[3] The physicochemical composition of pollen may be affected by other conditions such as collecting manner (bee or manually collected) and storage conditions.[4] The proportions of different constituents can also vary due to the time, the season of collection and the location of the plant from which pollen was

gathered.[5]

Pollen contains bioactive molecules such as polyphenolic compounds and flavonoids, well-known by their antioxidant capacity which contributes in the antiaging, anticarcinogen, anti-inflammatory and cardioprotective effects.[2,6]

Throughout history and around Arab world, date palm pollen (DPP) has been used in folk medicine to enhance fertility. Investigators showed a great improvement of the reproductive system of adult male rate, rabbit, and human being after being treated with DPP.[7-9] On the other hand, pollen has been used in food industry as a dietary supplement in yogurt, for example.[10] However, the latter study was only based on the antioxidant and antimicrobial activities of honey bee collected pollen. Then, to the best of our knowledge, there are no reports related to other properties of date palm pollen that could affect the quality of food systems such as surface and thermal properties.

Tunisia is considered as one of the date-producing countries counting around 5.4 million date palm trees. In the region of Sfax from Tunisia, many male date trees are discarded since every 50 female trees require 1 male tree as a pollen source.[11] Hence, the aim of this study is to add value to this discarded product. As a first step, we will focus on investigating the morphology, the physico-chemical, the surface and the antioxidant properties as well as the nutritional value of Tunisian date palm pollen. Then, we will discuss the possibility of a further cracking of this typical product to create novel ingredients acting as a bio-surfactant and replacing the synthetic antioxidants at the same time. Thus and as far as we know, this present work will be the first to relate physical to chemical properties in order to discuss the storage conditions, the way of processing and the probable behavior of DPP in agri-food and pharmaceutical field.

MATERIALS AND METHODS

Raw Material

Date Palm Pollen was manually collected from male date palm trees variety Deglet Nour aged at least 10-years, April 2015, Sfax, Tunisia. Spathes were gently shaken to separate pollen flour from flowers then sieved to remove residual particle and directly frozen in a watertight container at -20 °C for further uses.

DPP was analyzed for their properties, and all analytical determinations were performed in triplicate. Values were expressed as the mean±standard deviation.

Physico-Chemical Properties of Date Palm Pollen

Dry matter and mineral content: Dry matter (DM) and mineral content were determined according to the Association of Official Analytical Chemists.[12]

Proteins: Protein concentration was determined using a Dumas Elementar Rapid N cube 161 15054 (Donaustrasse, Germany). Total protein was calculated using a nitrogen conversion factor of 6.25.[13]

Fats: Fats were determined using an automated Soxtherm S 306 AK (GERHARDT, Germany) equipped with six soxhlet posts and a command unit. DPP was hydrolyzed with the standard method.[12]

Sugars content: DPP (200 mg) were extracted using 20 mL of ethanol (80%). The mixture was magnetically stirred for 30 min then centrifuged 10 min at 10000 rpm.[2] Twenty µL of filtered supernatant were analyzed with high-performance liquid chromatography (HPLC) equipped with Aminex HPX-87H column whose temperature was maintained at 40 °C. The mobile phase was sulfuric acid 3 mM in water and the flow rate was fixed at 0.4 mL/min. Glucose, fructose, and sucrose were identified and quantified using standards.

Amino acid analysis: The amino acid composition was assessed using HPLC. The sample was treated as described by Ghribi, Gafsi, Blecker, Danthine, Attia and Besbes.[14] The HPLC system (biochrom) was equipped with a UV-vis detector with two wavelengths, 440 nm, and 570 nm for the proline and the other amino acids, respectively, and a cation exchange column (200×4.6 mm). Cysteine and methionine, sulfur-containing amino acids, were quantified after a pre-hydrolysis oxidation with performic acid. The contents of the different obtained amino acids were expressed as g/100 g protein.

Water activity and pH: Water activity was measured at 20 °C with a Novasina, lab swift aw-meter, Switzerland.

A 1:4 (w/v) suspension of DPP in distilled water was prepared and pH was measured with a pH meter (827 pH lab Metrohm, Switzerland).

Color: The Cie Lab parameters (L*, a*, b*) were determined using a color flex EZ (Hunterlab, USA), calibrated with black and white tile. The L* value is a measure of lightness, ranging from 0 (black) to 100 (white); the a* value ranges from -100 (green) to +100 (red) and the b* value ranges from -100 (blue) to +100 (yellow). For Hue color index, 0° or 360° represents red and 90°,180°, and 270° represents yellow, green and blue, respectively.

The Hue angle (h°) and Chroma or intensity (C*) were calculated according to the following equations:

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$h° = \text{arc tang}\ (b^*/a^*)$$

Particle Size

The particle size distribution was measured using a laser light diffraction instrument (Malvern Mastersizer 2000. UK). The particle size that was expressed as d (0.1) and d (0.5). d (0.5) corresponds to the mean particle size for which 50% of the sample volume is below.

X-Ray Diffraction

A D8-Advance Diffractometer (Brüker, Germany) (k Cu = 1.54178 Å, 40 kV, 30 mA) equipped with a Lynxeye detector (Brüker, Germany) was used to study the (XRD) patterns of DPP sample. The data were collected in the 2θ ranges 5-40° with a step size of 0.02° and a counting time of 0.5 s/step.[14]

Scanning Electron Microscopy (SEM)

DPP scanning electron microscopy images were gathered using a scanning electron microscope (JSM-5400, JEOL, Japan). Pollen flour was placed on a copper holder and coated with a fine gold layer using fine coat, JFC-1100 E, Ion sputtering device, JEOL, Japan. Then, the sample was observed at an accelerating voltage of 15 KV and at 500, 2000 and 5000-fold magnifications.

Bioactive Compounds of Date Palm Pollen

Total phenolic: 2 g of DPP were mixed with 20 mL of acetone (50%) and the mixture was shaken 2 h at 25 °C, then centrifuged 20 min at 4500 rpm. The extraction was repeated twice to enhance polyphenols extraction.[15]

The Folin-Ciocâlteu method was used to determine the total phenolic content. The extract (500 µL) was transferred into a tube containing 2.5 mL of solution Folin-Ciocâlteu 1:10 in water. Two mL of sodium carbonate solution 7.5% (w/v) was added. The tubes were kept at room temperature for 15 min. Then, the absorbance was measured at 765 nm against distilled water as the blank sample.[16]

The total polyphenol content was expressed as Galic acid equivalents (GAE) in mg/100 DPP. Galic acid was used to obtain standard curve with concentrations varying between 0-50 mg/L.

Flavonoids: The previously described extract (250 µL) was transferred into a tube containing 1 mL distilled water and 150 µL $NaNO_2$ 15 % (w/v). Six min later, 75 µL $AlCl_3$ 10 % (w/v) were added. Finally, 1 mL 1 mol/L NaOH was added and the final volume was made up to 2.5 mL of distilled water. After 15 min incubation, the absorbance was measured at 510 nm against distilled water as the blank sample. Flavonoids content was expressed as quercetin equivalents (EQ) in mg/100 g DPP.[17]

Nitrogen Solubility Index

DPP flour was dispersed in distilled water and pH was adjusted to value 2-12 using 1 mol/L NaOH or 1 mol/L HCl. Dispersions were shaken 30 min at 25 °C, and finally centrifuged 5000 rpm for 15 min. Protein content in the supernatant was determined using Bradford method. Bovine serum albumin (BSA) was used to obtain standard curve from 20 to 200 µg/mL. The absorbance was measured at 595 nm. Solubility was calculated as the percent of protein in supernatant to the initial protein content in the sample.

Zeta Potential

A 0.1 g/100 mL protein dispersion from pollen was prepared prior to the measurement of the surface charge using a Delsa Nano C Instrument (Malvern Instruments, Westborough, MA, USA). The isoelectric point corresponds to the pH where the surface charge is null.

Surface Tension Measurement

A 1 g/100 mL DPP aqueous dispersion was prepared for the measurement of surface tension using an automated drop volume Tensiometer TVT1 (Lauda, Germany). The measurements were in dynamic mode with a syringe volume 2.5 mL, a drop creation time from 0.07 to 0.8 s/µL and at 25±0.5 °C. The lifetime of the drops was measured as a function of their volume, which made it possible to calculate the surface tension.[18]

Thermal Properties of Date Palm Pollen

Differential scanning calorimetry: Differential scanning calorimetry (DSC) was performed on DPP. Heat flow was recorded during heating from -50 and 250 °C at a scan rate of 5 °C/min with Thermal Analysis Instruments Q1000 DSC (TA DSC Q1000, New Castle, DE, USA). The sample was placed in hermetic aluminum-pans and an empty one having an equal mass of 0.1 mg was used as a reference. The temperature was calibrated with two standards (Indium, T_{onset}: 156.6 °C, DH: 28.7 J/g) and (Eicosane, T_{onset}: 36.8 °C, DH: 247.4 J/g). Specific heat capacity (Cp) was calibrated using a Sapphire. The cell was purged with Nitrogen 50 mL/min. The analyzed sample mass was about 2 mg and the experiment was carried out in triplicate.

Thermogravimetric analysis: The thermogravimetric analyzer (Mettler Toledo DSC/TGA 1 star system) was used to measure weight change during heating the sample from 25 to 800 °C with a step rate of 5 °C/min. DPP (10 mg) was placed in a ceramic pan. The Nitrogen gas flow rate was kept constant at 35 mL/min. The experiments were performed in triplicate to test the repeatability of the device. Data was collected automatically to get the weight loss rate curve.

RESULTS AND DISCUSSION

Physico-Chemical Composition

The physicochemical composition of DPP, presented in Table 1, showed that the moisture content was 30.31 g/100 g, which was comparable to the value reported for Egyptian palm pollen grains (29 g/100 g) by Hassan.[1] On the other hand, DPP was characterized by a neutral pH (6.31) and a high water activity equal to 0.898. These facts required either an immediate freezing or a rapid drying in order to prevent any microbial contamination. In this study, DPP has been frozen in its natural form for further analyses.

Table 1. *Physico-Chemical Properties of Date Palm Pollen*

Components	Values
Moisture (%)	30.31±0.86
aw	0.898±0.001
pH	6.31±0.01
Ash % (DM)	6.16±0.36
Protein % (DM)	38.18±0.60
Fat % (DM)	10.24±0.19
Total sugars % (DM)	
Glucose % (DM)	3.66
Fructose % (DM)	4.48
Sucrose % (DM)	10.08
Cie Lab parameters	
L*	84.37±0.09
a*	-1.11±0.02
b*	18.51±0.15
C*	18.55±0.15
h°	86.57±0.08
Particle size (µm)	
d (0,1)	12.75±0.12
d (0,5)	28.80±0.64
Total phenols (mg AG/100 g)	909.30±1.12
Bioactive compounds	
Flavonoids (mg EQ/100 g)	4.31±0.26

DM: Dry Matter, All the data are expressed as mean±SD and are the mean of three replicates.

The major component of DPP was proteins (38.18 g/100 g DM) (Table 1). This result was higher than that found by Hassan.[1] for date palm pollen (31.11 g/100 g DM) but similar to that found by Khider et al10 for Egyptian bee-collected date palm pollen (38.06 g/100 g DM). However, it is lower than the value given by Human et al[4] for the pollen of Aloe great head ii var. davyana (50.8 g/100 g DM). Thus, we may conclude that protein content can be related to the pollen source, the origin of vegetable material and climatic conditions.[10] From data collected, it was clear that DPP is a potential source of proteins that can be useful in agri-food and pharmaceutical fields.

DPP presented a relatively low amount of fats (10.24 g/100 g DM) when compared with the results of Hassan,1 which showed that the fat content of DPP was 21 g/100 g DM. According to Hassan,[1] this fraction contains various saturated, monounsaturated and polyunsaturated fatty acids and the predominant fatty acids of date palm pollen grain were palmitic (C16:0), linoleic (C18:2) and myristic (C14:0) acids.

DPP can be considered as a rich source of soluble sugars (18.22 g/100 g DM). Sucrose (10.08 g/100 g DM) was detected as the major sugar in the DPP sample, followed by fructose (4.48 g/100 g DM) and finally glucose (3.66 g/100 g DM). Hence, the physicochemical composition of DPP is an indicator of its high nutritive quality.

Concerning color coordinates, DPP powder was characterized by a high value of lightness (L*) equal to 84.37. The coordinate b* indicating the yellow color was equal to 18.51 and the coordinate a* measuring the red color was -1.11 (Table 1). From these values, DPP was a bright yellow flour. Therefore, the addition of DPP in food will not be the origin of any undesirable color. DPP seems to be a suitable food ingredient that guarantees a favorable organoleptic quality of food system.

Particle size characteristics revealed that DPP was composed of small particles. In fact, 10% and 50% of the particle do not exceed 28.80 µm and 12.75 µm, respectively. This size promotes functional properties such as water and oil holding capacities since small particles allow more contact with fluids.[19]

Amino Acid Analysis

The amino acid composition of the current studied DPP was presented in Table 2. The essential amino acids (Valine, Histidine, Isoleucine, leucine, lysine, methionine, phenylalanine, threonine and tryptophane) presented around 39% of the total detected amino acids in the analyzed sample, which indicated the great nutritional value of DPP. However, our value was lower than that reported for Egyptian date palm pollen (47%).[1] This slight dif ference was due to the variations observed in histidine, leucine, lysine and methionine content. Compared with other type of pollen, it was clear that DPP exhibited a relatively high amount of essential amino acids, followed by fresh Aloe great head ii var. davyana pollen (33%),[4] hardy kiwi pollen (30%) and finally oak pollen (23%).[3] On the other hand, the observed percentages remained important for human nutrition since they exceeded those reported by WHO/FAO/UNU (2007) (Table 1).

Table 2. *Amino-Acid Composition of Date Palm Pollen*

Amino acids (g/100 g protein)	DPP	WHO/FAO/UNU (2007)
Valine	5.16	3.9
Histidine	2.53	1.5
Isoleucine	4.37	3
Leucine	8.35	5.9
Lysine	7.73	4.5
Methionine	2.37	-
Phenylalanine	4.25	3.8
Threonine	4.70	2.3
Total essential amino acids	39.46	-
Tyrosine	3.46	-
Arginine	5.77	-
Alanine	6.48	-
Aspartic Acid	10.41	-
Glutamic Acid	13.23	-
Glycine	5.00	-
Cysteine	1.11	-
Serine	5.74	-
Total Non essential amino acids	51.20	-
Total sulfur amino acids	3.48	-
E/T (%)	43.53	-

As can be observed from the obtained results, among seventeen defined amino acids, glutamic acid (13.23%) was the major one followed by aspartic acid (10.41%), leucine (8.35%), lysine (7.73%) and alanine (6.48%). Glutamic and aspartic acids are responsible for a palatable taste, they are also considered as medicinal amino acid since aspartic acid has an anti-fatigue, antitussive and expectorant effect and glutamic acid is the most abundant free amino acid in skeletal muscle, it plays an important role in the synthesis of purines and pyrimidines by donating nitrogen.[20] The second group of amino acids was present in less percentage. Examples of these amino acids are arginine, valine, glycine, serine, threonine, phenylalanine and isoleucine, whose contents ranged between 3.46% and 5.77%. Alanine and glycine have a sweet taste. Besides, glycine is one of the major components of human skin collagen. It interferes with other essential amino acids to form a polypeptide that would promote regrowth and tissue healing, while arginine stimulates the secretion of pituitary and pancreas gland hormones, enhancing immunity and reducing the tumor protein synthesis and growth.[20] Moderate amounts of other amino acids were detected, such as histidine (2.53%), methionine (2.37%) and cysteine (1.11%). It was worthy to note that cysteine and methionine were the two least noted amino acids in other types of pollen.[3,4] Then, we might deduce that the amino acid composition depends on pollen's origin as well as the climatic and nutritional conditions of the plants on which the pollen matures.[1] Therefore, DPP could be used as a raw material to improve food's flavor and nutritional quality.

Bioactive Compounds

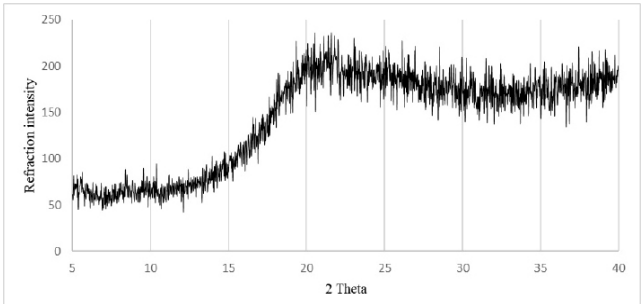
The obtained results (Table 1) showed that the phenol content of DPP is 909.30 mg EAG/100 g DM, which was in accordance with the data provided by Žilić et al[2] since maize pollen presented an average of 889.02 mg EAG/100 g. This value proved that DPP can be considered as a rich source of natural bioactive compounds that can be used to prevent the oxidation of many food products. Such data could be of great interest for consumers, since phenolic substances act as an antioxidant, and present multiple biological effects, including the reduction of the risk of heart disease, cancer, cataracts.[21] These compounds also prevent the oxidation of LDL lipoprotein, platelet aggregation and damage to red blood cells.[22]

Additionally, DPP had a very low content of flavonoids 4.31 mg EQ/ 100 g compared to date palm pollen 266 mg EQ/ 100 g and Mimosa pollen 548 EQ/100 g.[23] This difference could be explained by the fact that the total phenolic and flavonoids contents could be influenced by geographical origin, storage and drying procedure.[24] In addition, solvents used for the extraction process and its polarity could play a major role in increasing phenolic compounds recovery.[15]

X-Ray Diffraction

X-Ray diffraction (XRD) was performed to study DPP structure. Figure 1 revealed that XRD patterns did not show any band over the 2θ ranges 5-40°. The DPP had a dominant amorphous halo. Such amorphous structure may cause stability problems, while preserving DPP powder. However, it leads to better techno-functional properties when compared with crystalized flours. Thus, it is important to store DPP in a water-air tight container to preserve its quality.[14]

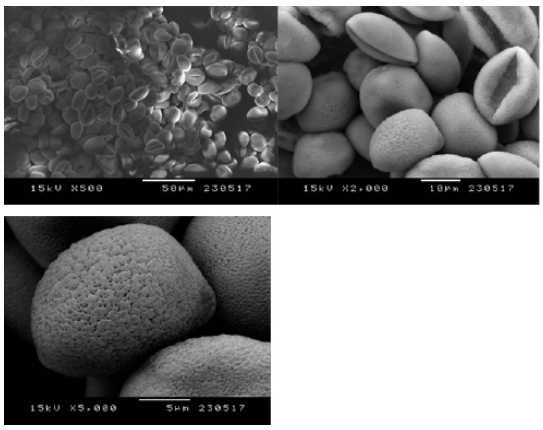
Figure 1. *X-Ray Diffraction Pattern of Date Palm Pollen*



Pollen Morphology by Scanning Electron Microscopy

Pollen morphology is an expression of its genome whose variability may be utilized in species identification.[25] Thus, DPP was submitted to scanning electron microscopy in order to investigate pollen's structure. The obtained SEM images proved that the morphology of the currently studied pollen was similar to that presented by Bishr and Desoukey.[5] In fact, the SEM analysis of DPP (Figure 2) showed monad, elliptical and bilateral particles with small size ranging between 10 and 25 µm. The DPP grains were characterized by one aperture from which the pollen tube grows, and many pores of various diameter ornamenting the exine surface, allowing the exchange of different substances with the outer environment. Exine's characteristics have been used in some studies to allow molecules to penetrate in the pollen grain. In fact, Atwe et al[26] demonstrated that the pollen grains of *Lycopodium Clavatum* can be evacuated through exine, which provides a clean spore filled with vaccine molecules for oral vaccination.

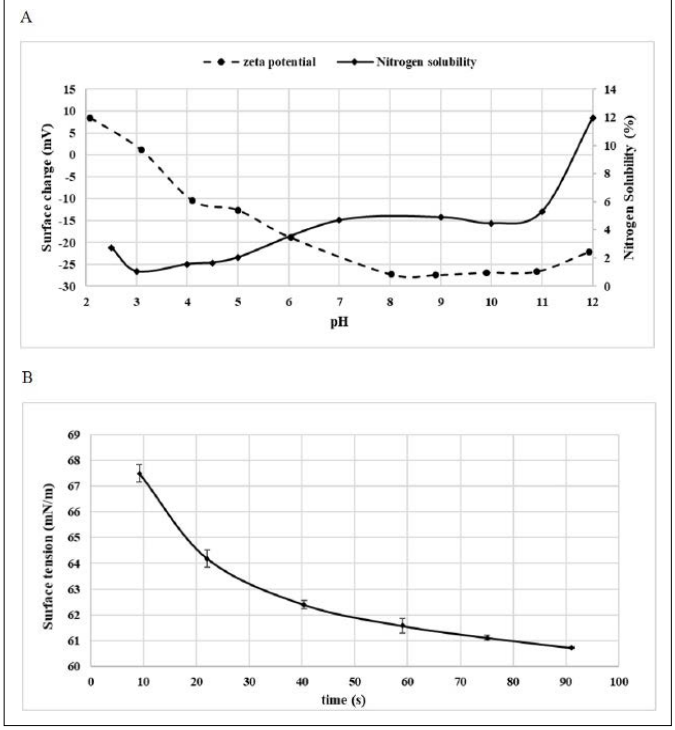
Figure 2. *Scanning Electron Microscopy Images of Date Palm Pollen*



Nitrogen Solubility Index

DPP solubility, as well as its surface charge, were presented in Figure 3A. Nitrogen solubility depends on suspension's pH, it increased when pH increased. Indeed, it was minimal (1%) at pH 3 and maximal (12%) at pH 12 (Figure 3A). These values were very low when compared with other flour's solubility. For example, Wani et al[27] studied the protein solubility of Indian kidney bean flour and found that the minimum of solubility (7.1%) occurred at pH 4 near the isoelectric point and the maximum was at pH 10 with an average of 96.55%. This low solubility value could be attributed to the structure of pollen grain which is composed of two layers, an outer exine, and an inner intine. The exine is composed of a strong biopolymer called sporopollenin which is resistant to acetolysis and high temperature while the intine is mostly made of cellulose and pectin.[26]

Figure 3. *Surface Properties of Date Palm Pollen* ***A:*** *Effect of pH on the Zeta Potential and Nitrogen Solubility,* ***B:*** *Surface Tension (1% Aqueous Suspension)*



Zeta Potential

Zeta potential analysis (Figure 3A) showed that the surface charge of DPP flour also depends on pH. Indeed, the more the pH was alkaline the more the surface charge was negative. This fact led to less attraction between proteins and increased solubility. The surface charge was the least negative at pH 3, which corresponded to the isoelectric point where the least solubility value was noticed.[28] The solubility profile could be exploited in further protein extraction.

Surface Tension Analysis

The ability of 1 g/100 mL DPP flour aqueous suspension to reduce the interfacial tension was studied in Figure 3B. As can be observed from the figure, the curve could be divided into two phases, a first one characterized by a sharp decrease in the surface tension from 67.5 mN/m to 61.5 mN/m and a second phase leading to the equilibrium of the surface tension near 60 mN/m (Figure 3B). These values were important when compared with values reported for date seed fibro protein extract Deglet Nour (1 g/100 mL protein concentration) that reduced the surface tension to 57.4 mN/m.[18] From all results, it was obvious that DPP could act as a surfactant thanks to its hydrophobic-hydrophilic proportion mainly existing in the protein fraction. In fact, this compound could reach the interface, leading to the reduction of interfacial tension. Lowering the surface tension may be the origin of a great foaming and emulsifying properties, which could improve food quality.[29]

Thermal Properties

Date palm pollen was submitted to the thermogravimetric technique in order to investigate the thermal behavior of the flour. Figure 4A showed six events characterized by different weight losses. While the first step occurred up to 100 °C and represented a weight loss of approximately 27%, the second event with a weight loss of 2.57% took place between 100 °C and 170 °C. Both steps were mainly attributed to the water loss. Indeed, the moisture determined with gravimetric method corresponds to 31%, which was the sum of the weight loss of both steps.

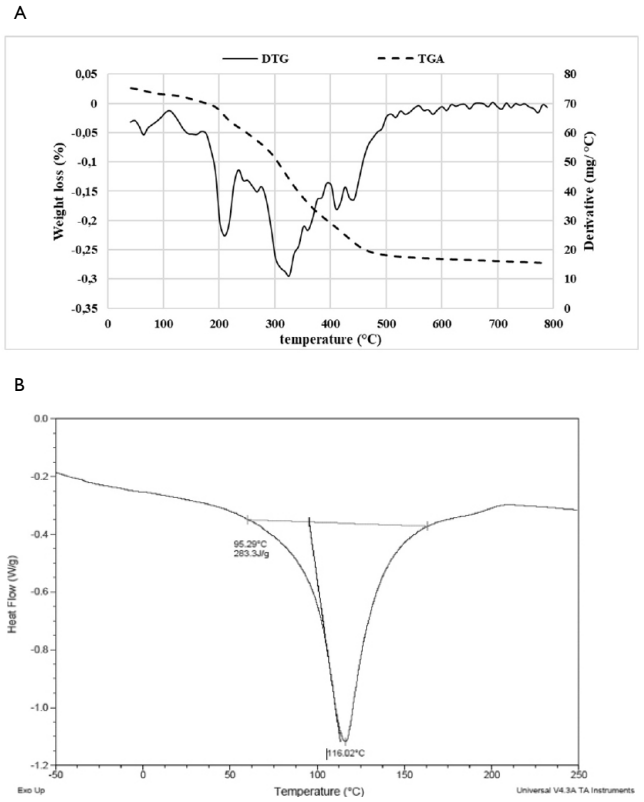
The third and fourth steps with a weight loss of 8.4% and 5.55%, respectively, were between 170 °C and 275 °C. This weight loss was due to successive thermal degradation reaction of carbohydrates and organic compounds, such as triterpenes, alkaloids, and flavonoids.[30]

In the fourth step, 27% of the total weight was lost between 275 °C and 400 °C. This was the result of a complete decomposition of carbohydrates, the beginning of the carbon formation and the elimination of volatile materials. The final event representing a weight loss of 11.7% refers to the oxidation of carbonaceous materials and the formation of ash.[30] From the collected data, we might conclude that the thermal decomposition of DPP flour occurred in a progressive way owing to the difference in the stability of different components against thermal treatment.

Differential scanning calorimetry (DSC) is an important tool that allows studying thermodynamics transitions. DSC Thermogram (Figure 4B) showed only one endothermic peak at 116.02 °C related to the transition of the protein from native to denatured form. This value was higher than the denaturation temperature of desi chickpea protein isolates which ranged between 98.5 and 99.8 °C.[31] However, oven and freeze-dried chickpea protein concentrates were more stable since the temperature of denaturation had an average of 131 °C, which was explained by the fact that proteins had lost their native form during drying.[14] DPP flour was conserved at a very low temperature (-20 °C) which preserved protein's quality. As a conclusion, DPP proteins seemed to be thermally stable during storage and in the different

food systems.

Figure 4. *Thermal Properties of Date Palm Pollen* ***A:*** *Thermogravimetric Curve,* ***B:*** *Differential Scanning Calorimetry Curve*



DTG: Derivative thermogravimetric analysis (speed of the weight loss); TGA: Thermogravimetric analysis (weight loss)

CONCLUSION

The obtained data clearly proved the substantial nutritional value of date palm pollen due to the exceptional content of protein, amino acids, phenols, and flavonoids. The amorphous structure determined using X-ray diffraction proved that DPP could present great techno-functional properties while stored in suitable conditions. Lowering the surface tension using DPP yields interesting results for using it as a novel efficient natural surfactant as a whole or by the extraction of protein fraction to improve the surfactant properties. Therefore, date palm pollen is recommended to be used as an ingredient in functional food to ameliorate its nutritional and organoleptic quality.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Product Development and Quality Evaluation of Biscuit and Ready-to-Eat Snack from Cowpea-wheat Flour Blends

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ABSTRACT

Aim
The research was conducted with the aim to develop biscuit and ready-to-eat snack product from cowpea flour incorporated with wheat flour.

Methods
The wheat and cowpea flour blends were prepared in five blending ratios including B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), and B5 (53:47); respectively. The D-optimal mixture design software was used for flour blend formulation. Biscuit and extrudate products from cowpea and wheat blends were analyzed. Quality characteristics parameter used for value-added products includes physical, functional, proximate, mineral and microbial quality. Furthermore, bioactive components and sensory quality evaluation were also investigated. The biscuit samples were prepared at a baking temperature of 205 °C for 10-minutes holding time. The extrudate samples were also manufactured at feed moisture (18 and 21%), barrel temperature (100, 110 and 120 °C) and screw speed (175 and 220 rpm).

Results
The result for crude protein analysis of biscuit (B3) and extrudate (Ex-3) samples was revealed that 15.972±0.125, and 15.915±0.251; respectively. The result for microbial quality analysis of biscuit (B3) and extrudate (Ex-3) samples was also shown as aerobic bacteria count of 44×10^{-5}, 42×10^{-5} and yeast and mold (un-detected); respectively. The highest overall sensory evaluation of biscuit (B3) and extrudate (Ex-3) samples score were found 7.6 and 7.14; respectively.

Conclusion
Based on quality evaluation parameters, 35% cowpea flour with 65% of wheat flour blending ratio reveled sensor acceptable for biscuit production and manufacturing of ready-to-eat snack.

Keywords
Biscuit; Cowpea; D-optimal mixture; Extrudate products; Wheat.

INTRODUCTION

Grain legumes are rich and less expensive sources of dietary proteins and water-soluble vitamins. Legumes contain twice as much protein as cereals and, except for the sulphur-containing amino acids (methionine and cystine which are adequate in cereals) the amino-acid profile of most legumes complement those of cereals.[1] Among the legumes, cowpea is believed to be the most widely grown, distributed and traded food commodity in Africa.[2]

Cowpea, like other grain legumes, is an important foodstuff in tropical and subtropical countries.[3] Nutrients provided by cowpea make it extremely valuable where many people cannot afford proteins from animal sources such as meat and fish.[4] Cowpea also cultivated in Ethiopia and use for traditional food preparation and animal feed as a meat source. It is well adapted to the stressful growing condition of the tropics and has excellent nutritional qualities.

Extrusion combines a number of unit operation i.e. mixing, cooking, shearing, puffing, final shaping and drying in one energy efficient rapid continues process[5] and can be used to reduce a wide variety of starchy foods including snacks, ready to eat (RTE), confectioners, and extruded crispbreads. This process of high-temperature short time extrusion brings gelatinization of starch, denaturation of protein, modification of lipid and inactivation of enzymes, microbes and many anti-nutritional factors.[6] The aim of this research was to develop and evaluate the substitution of wheat by cowpea and then investigate the quality of value-added products.

MATERIALS AND METHODS

Sample Collection, Transportation, Preparation and Storage

Cowpea samples were collected from Measo Agricultural Research and Satellite Center, and hard wheat variety was obtained from Debrezeit Agricultural Research Center. All research samples were packed with polyethylene plastic bags and stored at dry condition in the room temperature at food engineering laboratory for further use.

Blend Formulation

The cowpea grains were steeped in tap water at 28 °C and decorticated. De-hull cowpea grain was dried at 52 °C and milled with laboratory miller (CIT-LDM-15, USA) sieve size of 750 µm.[7] Blended flour for cowpea and wheat flour were prepared as B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0)(wheat to cowpea flour), respectively using D-optimal mixture software.

Biscuit Products Development

The various blends formulated from a mixture of cowpea and wheat flours were mixed separately with the same quantity of other ingredients (350 g flour, 115 g sugar, 50 mL baking fat, 10 g milk powder, 0.25 mL vanilla, 1.75 g salt, 71 mL water and baking powder 2 g) adapted from Gamal et al.[8] The fat was creamed with sugar until fluffy, and other ingredients were also added. The batter was kneaded on a rolling table to acquire the desired thickness. The batter was later cut to square shape with the aid of knife cutter. It was baked in the oven at 205 °C for 10-minutes, cooled and packaged for further use.

Extrusion Process

The blended samples were also fed manually through a rotatable and screw operated conical hopper using Twin-screw extruder (Clextral, 42702 Firmly, France). The hopper, which was mounted vertically above the end of the extruder, equipped with a screw speed which was adjusted to 7 and 8 rpm and water pump stoke level adjusted to give 18 and 21% feed moisture; respectively. The blended flour was extruded at a screw speed of 175 and 200 rpm, barrel temperature (100, 110,120 °C), and feed moisture (18 and 21%) with 1.0 mm die diameter of extrusion. The barrel temperature at zone three was controlled with a temperature sensor probe fit which is controlled by cold water motorize pump. Experimental samples were collected when a steady state (constant temperature and torque) was achieved. The extrudate products were kept on aluminum foil with benches to dry. Finally, all samples were packaged in polyethylene bags for further analysis.

Analyses Methods

Flour quality analysis

pH of flour: The pH values of the blended flours were determined using the official association of analytical communities (AOAC) method.[9] About 20 g blended flour sample was put into 100 mL of distilled water and the supernatant was poured into another measuring cylinder. Finally, the pH of the homogenate was measured at room temperature with Jenway, UK, 3505 pH meter.

Water absorption (WAI) capacity: WAI capacity was determined using a method described by Solusulski.[10]

Foaming capacity and foam stability: Foaming capacity and foam stability was determined according to the method described by Ohizua ER et al.[11]

Bulk density and dispersibility of flour: According to Narayana K. and Narasinga Rao, bulk density of flour was determined with leveled flask tube which was weighed and filled with a sample to 5 mL by constant tapping until there was no further change in volume.[12] Bulk density was calculated as the mean of the triplicate determination of the weight determined by difference per unit volume of the sample. Dispersibility of blended flour in water was also determined with accurately weighed 10 g of each flour sample was placed in a 100 mL measuring cylinder followed by addition of distilled water up to the 100 mL mark. The sample was vigorously stirred, mixed and next allowed to settle for 3 hr. The volume of the settled particles was recorded and subtracted from 100 to give a difference which is considered as percentage dispersibility of each blended flours.[13]

Proximate composition analysis: Moisture content, crude fat, crude protein, ash, the crude fiber of sample was determined according to AOAC official method number 925.10, 920.39, 992.15 (39.1.16), 923.03 (32.1.05) and 978.10; respectively.[9]

Mineral concentration: The method described by AOAC official method number 984.27, using atomic absorption spectrophotometer (AAS) (Shimadzu, 7000, Japan) was used for mineral contents determination.[9] The minerals determine were iron, magnesium, calcium and zinc at 248.52 λ, 285.49 λ, 422.81 λ, and 214.11 λ; respectively.

Bioactive compounds: Phytate content was determined based on complex formation of phytic acid and Fe (III)-ion at pH 1-2, after extraction in 1.2% hydrogen chloride (HCl) solution containing 10% Na_2SO_4 as described by Thompson and Erdman (1980).

An excess of Fe (III)-ion present in the solution would react with thiocyanate ion to form a characteristic pink complex, Fe (SCN). The optical density at 465 nm was measured,[14] and an inverse linear relation was found for phytate concentration from 40 to 200 nmol/L.

About 0.2 g of the sample was placed in 100 mL conical flask to determined tannin. Ten milliliters 1% HCl in methanol (v/v.) was added. The contents were mechanically shaken for 20 minutes and centrifuged at 2500 rpm for 5-minutes. One milliliter of supernatant was pipetted into a test tube and 5 mL of vanillin-HCl regent (mixing equal volume of 8% concentrated HCl in methanol and 1% vanillin in methanol) were added according to price et al.[15] The optical density was read using a colorimeter. At 500 nm after 20-minutes' incubation at 30 °C, a blank sample was carried out with each run of samples. A standard curve was prepared to express the result as catechin equivalents, i.e. amount of catechin equivalent expressed in mg per mL which gives color intensity equivalent to that given by tannin after correcting for blank. Then the tannins amount calculate as follows:

$$\text{Tannins (mg/100 g)} = \text{Ad} \times \text{wt.s} \times 10 \quad \text{Eq(1)}$$

Biscuit quality assessment: Bulk density, WAI, and water solubility index (WSI) were determined using a method described by Solusulski.[10] Fracture strength of biscuits was measured with the Texture Analyzer (Model TA-XT2i, Stable Microsystems, Haslemere, U.K, 2014) using a 2-point Bending Rig and 5 kg load cell. The distance between the two beams was 40 mm. Another identical beam was brought down at a pre-test speed of 1.0 mm/s, test speed of 3.0 mm/s, post-test speed of 10.0 mm/s. The downward movement was continued until the biscuit broken. The peak force that used to crash was reported as fracture strength expressed in Newton.

Quality evaluation of ready-to-eat extruded products Bulk density (ϱ_b): The bulk density of the extruded product was calculated after measuring weight, length, and diameter of the extrudate immediately after extrusion.[16]

$$(\varrho_b) = \frac{(4 \times \text{wt of extrudate(g)})}{(\pi \times \text{Diametr of extrudate}^2 \times \text{Le})} \quad \text{Eq(2)}$$

Where: Le=length of extrudate

Degree of expansion: The diameter of the extrudate was measured with an electronic digital caliper and also the weight of extrudate was measured by a digital balance. The degree of expansion (expansion ratio) (diametrically) was defined as the ratio of the diameter of the extrudate to the diameter of the die hole.[16]

Water absorption (WAI) and water solubility index (WSI): WAI and WSI were determined using a method described by Solusulski.[10]

$$\text{WAI} = \frac{((\text{Weight of tube} + \text{Wrac-Wec}) \times 100)}{(\text{Weight of sample})} \quad \text{Eq(3)}$$

$$\text{WSI} = \frac{((\text{Wcad-Wec}) \times 100)}{(\text{Weight of sample})} \quad \text{Eq(4)}$$

Where: Wrac=Weight of residue after centrifuge
Wcad=Weight of crucible after drying
Wec=Weight of empty crucible

Microbiological Analysis

The microbiological analysis (total aerobic bacteria, yeast, and molds) sample was also carried out.[17] Plate count agar (PCA) and potato dextrose agar (PDA) was used for enumeration of bacteria, yeast, and mold; respectively.

Sensory Quality Attributes

The biscuit and extrudate samples with controls were presented separately for 36 semi-trained panelists in food engineering laboratory for sensory acceptance tests. All samples were tested by each individual of untrained panelist for color, appearance, flavor/aroma, crispness, and overall acceptability. The samples were evaluated on nine (9) hedonic scale method as described by Ihekoronye et al.[18]

Experiential Research Design and Statistical Data Analysis

A completely randomized design experiment was applied in the evaluation of grain quality analysis, flour quality analysis, and biscuit samples quality evaluation with one factor (blending ratio) in order to minimize unknown and uncontrolled factors or variables.

For the extrudate also used a mixed general full factorial design (3×2×2) experiment. Mixed general factorial design experiment was used to study the main factors affecting the drying process in the experiment namely, barrel temperature (100 °C, 110 °C and 120 °C), feed moisture (18% and 21%) and screw speed (175 rpm and 220 rpm) and interaction between the main factors at 95% confidence interval for the final extruded product. One-way analysis of variance (ANOVA) was used to for comparison of means ($p<0.05$). All data were expressed mean with standard deviation and list of significance difference (LSD) analyzed using statistical package for social scientists 20.0 (SPSS), and design expert version 7.0 software.

RESULTS AND DISCUSSION

Physical Properties of Cowpea Grains

The physical properties of the bole cowpea grains such as density, WAI, hydration capacity and index, swelling capacity and index, hydration coefficient, swelling coefficient, mass of seed coat,

soak ability and cooking time studies were 1.200 g /mL, 93.27%, 0.151 g/seed, 1.507, 0.16 mL/seed, 0.800, 2.195, 2.255, 0.402/10 g, 100% and 13-minutes; respectively (Table 1).

Table 1. *Physical Properties of Cowpea Grains*

Physical Property	Mean±SD
100 seed/kernel mass (g)	17.185±0.460
100 seed/kernel volume (mL)	14.333±0.577
Seed density (g/mL)	1.200±0.056
Water absorption (%)	93.270±1.260
Hydration capacity (g/seed)	0.151±0.008
Hydration index	1.507±0.341
Swelling capacity (mL/seed)	0.160±0.029
Swelling index	0.800±0.144
Hydration coefficient	2.195±0.059
Swelling coefficient	2.255±0.135
Mass of seed coat/10g	0.402±0.032
Seed soak ability (%)	100
Cooking time (min)	13±1

All results are expressed as means±standard deviation (SD) with triplicate determination

The physical properties of the seed are a useful tool to know to handle, transporting and processing equipment design and system as well as to determine the amount of water required for processing from raw material up to end products. The density of bole cowpea grain is a measure of hardness and heaviness which is related to machine operation, yield during flour production, water consumption in wet processing, power consumption, de-huller design, separator equipment design, and determine handling and storage material selection as well as equipment design. All these physical properties are used in predicting the grain machine operation condition.[19]

The amount of seed coat of bole cowpea is 4% (w/w), this indicate the nature of the cowpea is more solids and less waste (hull) which has advantages in flour production yield. Hydration capacity and swelling capacity is also an important parameter in order to determine the cooking time and the energy required to process.[20] Higher values of hydration capacity and swelling capacity shows that less cooking time, which is better cooking quality and less energy consumption. Soak ability of bole cowpea was determined as 100%. These values show bole cowpea has no non-soak ability seeds which indicate a higher flour yield and best quality of seed. Cooking time also an important parameter in order to predict the amount of energy consumption as well as energy cost during processing.[20]

Physical Properties of Blended Flours

Cowpea flour and wheat flour blends B0, B1, B2, B3, B4, B5, and B6 of Physical properties studies were presented in below Table 2 pH, bulk density, and dispersibility of cowpea flour and its blends was range from 6.31 to 6.70, 0.60 to 0.79, and 71.50 to 76.00; respectively. The pH was indicating the nature of the flour which is around neutral pH zone and it's may need good packaging's and storage controlled condition to prevent microbial growth and flour damage. Bulk density also useful tool to related and determine to the design of storage equipment, transport and flow rates of the flour.[19] Bulk density mainly affected by internal moisture and particle size of the samples.[21] The coarse particle size of flour was a lower bulk density and fine particle size had a higher bulk density as well as a higher moisture content of flour may also increase bulk density due to a little incremental of flour weight. The cowpea flour has a higher bulk density 0.791±0.031, which means, it has a fine flour particle size and higher moisture content.

Table 2. *Physical Properties of Blended Flour Samples*

Sample Code	Physical Properties		
	pH	Bulk Density (g/mL)	Dispersbility (%)
B0	6.70±0.020^{a}	0.791±0.031^{a}	75.500±3.109^{a}
B1	6.34±0.010^{d}	0.693±0.035^{b}	68.500±2.121^{c}
B2	6.56±0.032^{b}	0.601±0.061^{c}	76.000±1.414^{a}
B3	6.49±0.015^{bc}	0.693±0.026^{bc}	73.500±2.121^{ab}
B4	6.44±0.026^{c}	0.773±0.009^{ab}	74.000±2.828^{ab}
B5	6.54±0.049^{b}	0.757±0.017^{ab}	73.500±0.707^{ab}
B6	6.31±0.015^{d}	0.688±0.025^{bc}	71.500±0.707^{bc}

$^{a-d}$*All results are expressed as means±standard deviation with triplicate determination. Values expressed with similar subscripts are not significantly different by Duncan multiple range Test across columns ($p<0.05$).*
Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Dispersibility blended flour showed that there was no significant difference between B0 (100% cowpea flour) and B2 (40% wheat and 60% cowpea flour). As well as there were no significant difference across the column among B3 (65% of wheat and 35% of cowpea flour), B4 (78% of wheat and 22% of cowpea flour) and B5 (53% of wheat and 47% of cowpea flour). But, there were a significant difference between B0 (100% cowpea flour), B1 (90% of wheat and 10% of cowpea flour) and B6 (100% of wheat flour). Dispersibility of B2 blended flour was higher values among the other, it shows that the flour has higher WAI and the dough will easily reconstitute to give fine consistency during mixing and baking in different baked products. Hundred percent of wheat flour dispersibility was lower than 100% of cowpea flour and its wheat and cowpea flour composite, however, all dispersibility values of blended flour has relatively higher, hence the dough was easily constituting during mixing.[22]

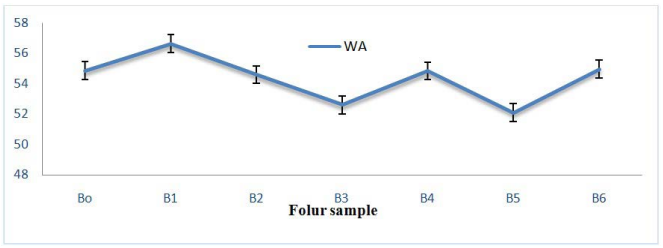
Functional Properties of Blended Flour

The WAI capacity of the blended flour was presented in Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Figure 1, WAI capacity higher in B1 and lower in B5 which is 56.649% and 52.100%, respectively. This result shows a significant difference and has an effect on yield and consistency of

flour dough. The WAI capacity of flour determined the amount of water absorbed and hold by starch granules and indicates the integrity of starch in aqueous phase dispersions. WAI capacity also related to hydrophilic properties of starch and protein in the flour and indicates a higher WAI capacity helps to improve food consistency and yield in food preparation.[23]

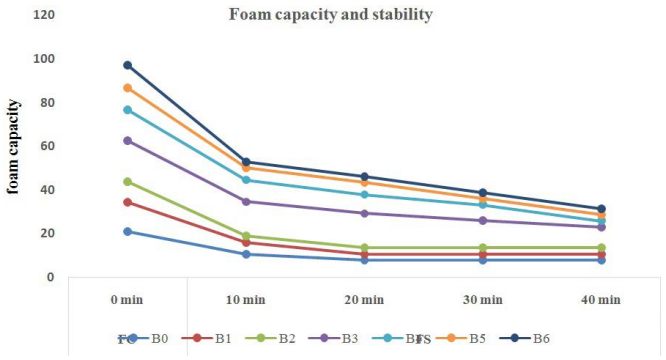
Figure 1. *Water Absorption Capacity of Blended Flour Samples*



Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Functional properties of the blended flour especially foam capacity and foam stability are dependent on the configuration of flexible protein molecules which have a good foaming capacity. But large conjugated globular protein molecules give low foam formation capacity.[24] Foaming properties are also dependent on the amount of carbohydrate present in the flour besides of water-dispersible protein.[23] This enables to form interfacial films on the upper surface and maintain air bubbles in suspension and slows down the rate of coalescence. Food ingredients with good foaming capacity and stability can be used in bakery products to improve texture, consistency, and appearance of foods.[25] This study shows that 100% cowpea flour (B0) and 65% of wheat and 35% cowpea blend flour (B3) had higher foam stability and capacity (Figure 2). Besides this, cowpea flour had higher foaming properties. Because, it contains free water dispersible nitrogen and carbohydrates. It plied an important role in bakery products to enhance sensory qualities. The pure cowpea flour foam capacity was 21.05% and foam stability was 7.89% after 40-minutes stability time. This finding is also in agreement with reported by Peter.[25] But, the concentration of the cowpea flour increased in wheat flour foam capacity and foam capacity was decreased.

Figure 2. *Foam Capacity (FC) and Foam Stability (FS) of Blended Flour Samples*



Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Proximate Analysis of Blended Flour

Legumes are very essential to provide plant protein and carbohydrate. Bole cowpea with wheat blended flour proximate compositions results were presented in Table 3. The amount of crude protein in each blending ratio has a significant change respect to the bole cowpea flour crude protein concentration. The amount of bole cowpea flour increased in blending, the concentration of crude protein also increased proportionally. According to Yadahally et al cowpea has a good source of crude protein.[26] Total energy of the flour had minimum value on cowpea flour and maximum value at the B5 (53% and 47%) of wheat and cowpea flour respectively. This is due to a higher amount of carbohydrate of wheat and higher amount of crude protein also in cowpea flour. The amount of moisture present in cowpea was minimum values than other bleeding flours because of flour particle size of cowpea was fine than the wheat flour.

The amount of crude fiber in pure cowpea flour recorded 2.05% and 3.13% (w/w) in pure wheat flour and in all blending flour, crude fiber was increased proportionally with increasing of the amount of wheat flour. This is due to the higher amount of fiber present in wheat grain and the fiber of cowpea grain was removed before preparation of blended flour. Ash content of pure

Table 3. *Proximate Composition Results of Blended Flour (Wheat and Cowpea)*

Code	Moisture (%)	Crude Fiber (%)	Fat (%)	Crude Protein (%)	Total Ash (%)	Carbohydrate (%)	Energy (kJ/100g)
B0	7.892±0.130^{d}	2.050±0.000^{d}	2.000±0.00^{b}	22.350±0.136^{a}	3.392±0.272^{a}	62.218±0.539^{e}	1506.46
B1	9.700±0.141^{ab}	3.170±0.254^{a}	2.000±0.000^{b}	13.323±0.093^{f}	1.399±0.275^{d}	70.407±0.255^{a}	1500.23
B2	8.981±0.257^{bc}	2.440±0.282^{c}	1.500±0.00^{c}	17.588±0.169^{b}	2.200±0.283^{b}	66.789±0.426^{d}	1504.94
B3	8.608±0.554^{dc}	2.715±0.007^{b}	1.500±0.000^{c}	16.277±0.132^{d}	1.996±0.005^{bc}	68.402±0.409^{c}	1512.29
B4	9.209±0.270^{bc}	2.840±0.155^{b}	2.000±0.000^{b}	14.903±0.127^{e}	1.444±0.785^{d}	69.603±0.543^{b}	1510.42
B5	10.490±1.569^{a}	2.340±0.099^{c}	1.000±0.00^{d}	16.889±0.267^{c}	1.602±0.562^{cd}	66.677±1.764^{d}	1490.55
B6	9.690±0.127^{ab}	3.313±0.035^{a}	2.250±0.004^{a}	12.687±0.124^{g}	1.402±0.279^{d}	70.904±0.318^{a}	1499.13

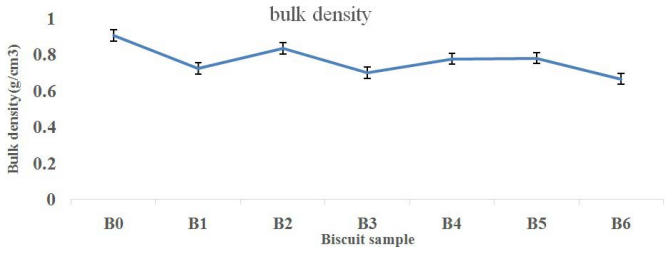
$^{a-g}$All values were means of duplicate determination±SD (Standard deviation) and expressed in dry basis, except moisture.
Values expressed with similar subscripts are not significantly different by Duncan multiple range Test across a column ($p<0.05$).
Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

and blended flour range from 1.399% in 90% and 10% of wheat and cowpea flour and 3.392% in 100% cowpea flour. The amount of ash present in the higher amount into pure cowpea flour, this is implying that besides of higher amount of carbohydrate and crude protein of cowpea flour, it has also a higher concentration of ash and it is an important implication of the presence of different minerals in cowpea flour. Whatever it is, cowpea flour is unique for cowpea flour-based product development in the bakery industry to enhance nutrition status in higher amount respect to wheat based products. The total carbohydrate in pure cowpea is 62.218% and 70.904% in pure wheat flour, but total carbohydrate of the blending is increased proportionally with incremental of wheat flour concentration in the flour. Proximate composition result of cowpea flour which is present in Table 3 similarly reported in Henshaw findings.[27]

Quality Evaluation of Biscuit

Bulk density: Bulk density of biscuit samples presented in Figure 3. The highest and lowest bulk density was observed in cowpea (B0) and wheat biscuit sample (B6) alone, respectively, from the seven samples which were prepared at a different blending ratio of flour. This study shows that the bulk density of each sample relies on between 0.906 g/cm^3 to 0.665 g/cm^3. Bulk density is an important parameter in the design and selection of packaging material. It is also an indication of the porosity of the product, wettability, and crispness. Furthermore, it also helps in infant feeding, if the product has less bulk density.[28] Biscuit sample which is developed from B3 (65% wheat and 35% of cowpea flour) has the smallest bulk density next to the control sample B6 (100 % wheat flour). The lowest bulk density means that it has a higher crispness, higher porosity and good product physical qualities.[25]

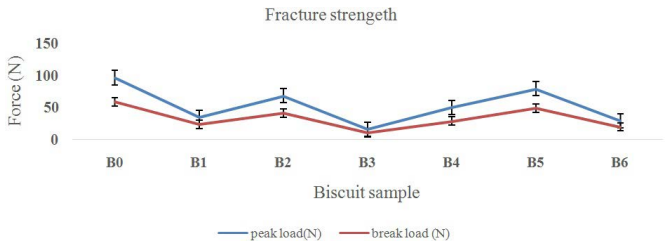
Figure 3. *Bulk Density of Biscuit Samples*



All results were expressed mean± SD of three independent determinations.
Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Fracture strength of biscuit: Fracture strength (break load) *versus* peak load of biscuit samples were plotted in the following Figure 4. A breaking load is a measure of force that required to splitting biscuit structure easily. B3, B1, B4 and B6 (control) have a minimum break load force beyond the other three biscuit samples. This indicates a direct relationship with human mouth feel (crispness). It means a lower break force required to break the biscuit sample directly represents a higher value in the crispness of the biscuit product. The fracture strength of biscuit B3 was lowest than the others. This may have observed due to less gluten content of cowpea flour. Similar findings also observed in buckwheat flour biscuit.[29] Which was decreasing in gluten content buckwheat flour, the fracture strength of biscuit was lowest. It implies that the biscuit sample has more crispness characteristics.

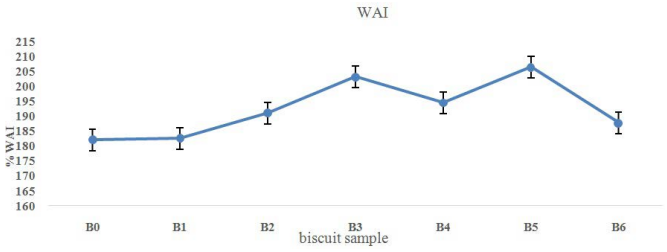
Figure 4. *The Fracture Strength (Peak and Break Load) of Biscuit Sample*



Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

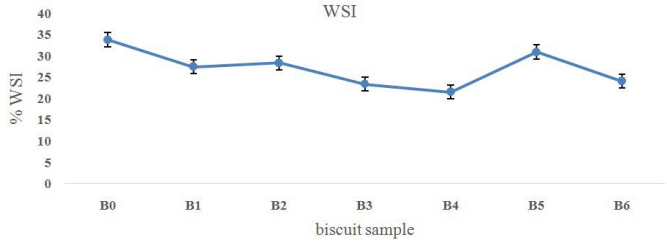
Water absorption and water solubility index (WAI & WSI): WAI index of biscuit samples was presented in Figure 5. The least and highest WAI of biscuit sample were found in sample B0 (100% of cowpea flour) and B5 (53% wheat, 47% cowpea flour). WAI index shows the quality of the biscuit sample which is soluble or not and starch was not converted into plastics natures or retrograde during oven baking. It is also an index of starch gelatinization or measures the amount of water absorbed by starch.[30]

Figure 5. *Water Absorption Index of Biscuit Samples*



Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

Figure 6. *Water Solubility Index of Biscuit Samples*



Where: B0 (0:100), B1 (90:10), B2 (40:60), B3 (65:35), B4 (78:22), B5 (53:47), B6 (100:0) (wheat to cowpea flour); respectively.

WSI of biscuit sample B0 (100% of cowpea flour) and B4 (78% of wheat and 22% cowpea flour) have a maximum and minimum values; respectively (Figure 6). WSI is also an important vehicle in infant feeding beyond adult age consumption.[28] It is also an indicator of degradation of a starch molecular component during processing. The higher baking temperature could increase the degree of starch gelatinization that could increase the amount of soluble starch resulting in an increase in WSI.[31]

Flake-extrudate Quality Analysis

Expansion ratio: Expansion ratio describes the degrees of puffing perpendicular to the extruder die which a sample as it exits the extruder. This phenomena's dependent on the viscosity and elastic properties of the dough in the extruder.[32] Expansion of extrudate is an important physical property of a snack and ready to eat food in food industries.[33]

The expansion ratio of an extruded product also was mainly dependent on the barrel temperature, screw speed and the interaction between screw speed and feed moisture of the extruder. ANOVA shows, this extruder parameter (barrel temperature, screw speed and the interaction between screw speed and feed moisture) significantly affected the wheat-cowpea extrudate.

The expansion ratio measured for all extruded samples of Ex-3 (65% wheat and 35% cowpea flour) ranged from 1.126 to 1.656. The expansion ratio of extrudate increase as barrel temperature increases, this is due to the enhancing of melting and gelatinization of starch component[34] that goes to the increment of volume of the extrudate and while decreasing the bulk density. As temperature increase too much, the gelatinization process may go to the weakening structure of starch before plasticization and expansion ratio would decline. According to Figure 7, expansion ratio increased gradually, while screw speed decreased, this is due to the energy of screw exerted on dough without sufficient time of gelatinization temperature in the extruder. Due to this, maximum screw speed the expansion ratio would be minimum and at minimum screw speed, the expansion ratio would be higher. The interaction effect of feed moisture and screw speed had a negative effect on expansion ratio (Figure 8), expansion ratio decrease as screw speed and feed moisture values increase from low to high level at constant barrel temperature. And that, screw speed decrease from higher to lower level the feed moisture effect on the Ex-3 extrudate goes to constant.

Bulk density: The effect of extruder parameter on extrudate bulk density is in Figure 9 on the contour surface plot. The extruder parameter (barrel temperature, feed moisture, and screw speed) significantly affected the bulk density of extrudate. Bulk density is related to expansion ratio which describes puffing character in all direction of the extrudate.[32,35] As bulk density decrease, indirectly expansion of extrudate would be increased. As shown below in the contour surface plot, feed moisture of the dough increases the bulk density of the extrudate increases, but when barrel temperature increases bulk density would decrease. During this, the feed moisture also decreases. This is due to that barrel temperature would be vaporized the lower feed moisture than the higher feed moisture easily from the melted and expanded product and forms pores (puff) structure. The formation of expanded products is associated with high pressure and temperature.[33] The increasing of feed moisture would decrease the temperature of the dough in the barrel zone between the screw and barrel. This would be a reduction of starch gelatinization and puff formation that leads to reduce the expansion of products. Therefore, the bulk density of the extrudate would increase as decreasing of expansion.

Screw speed also influences on the bulk density of extrudate. The screw speed increase means output rate increases, regarding of this exposure of dough to barrel temperature was insufficient to melt and gelatinize of protein and starch com-

Figure 7. *Effect of Screw Speed and Barrel Temperature on the Expansion Ratio of Extruded Product at Constant Moisture Content*

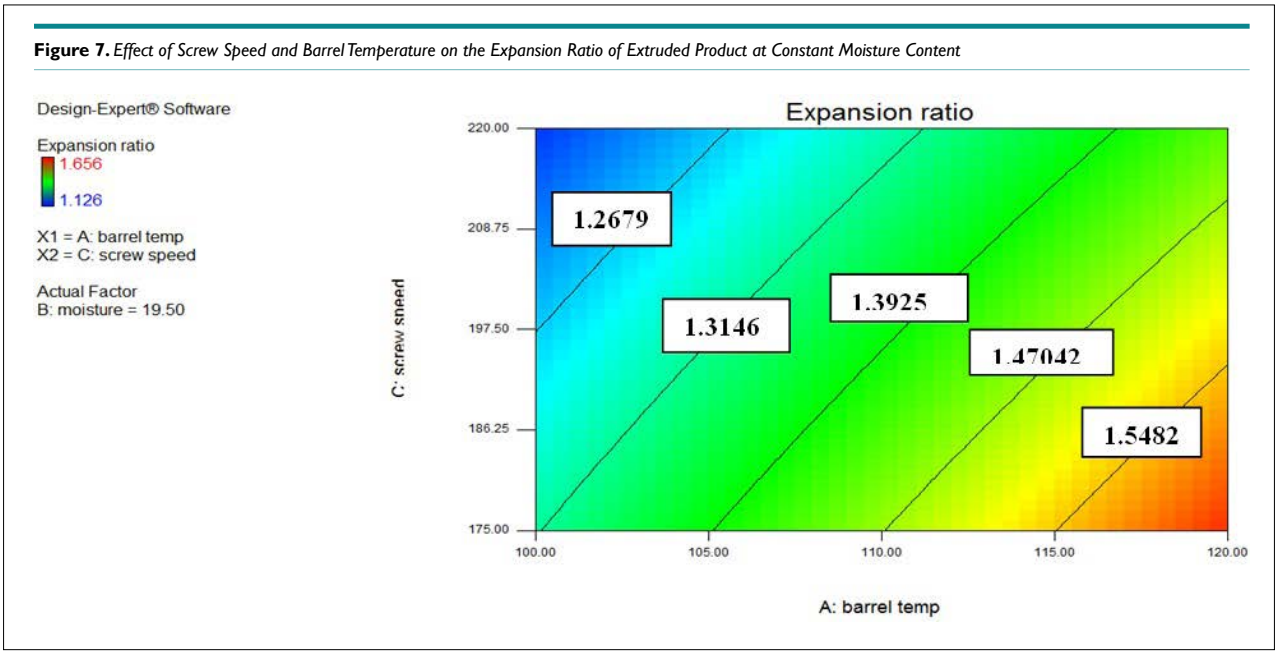


Figure 8. *The Interaction Effect of Screw Speed and Feed Moisture on Expansion Ratio of the Extrudate at Constant Barrel Temperature*

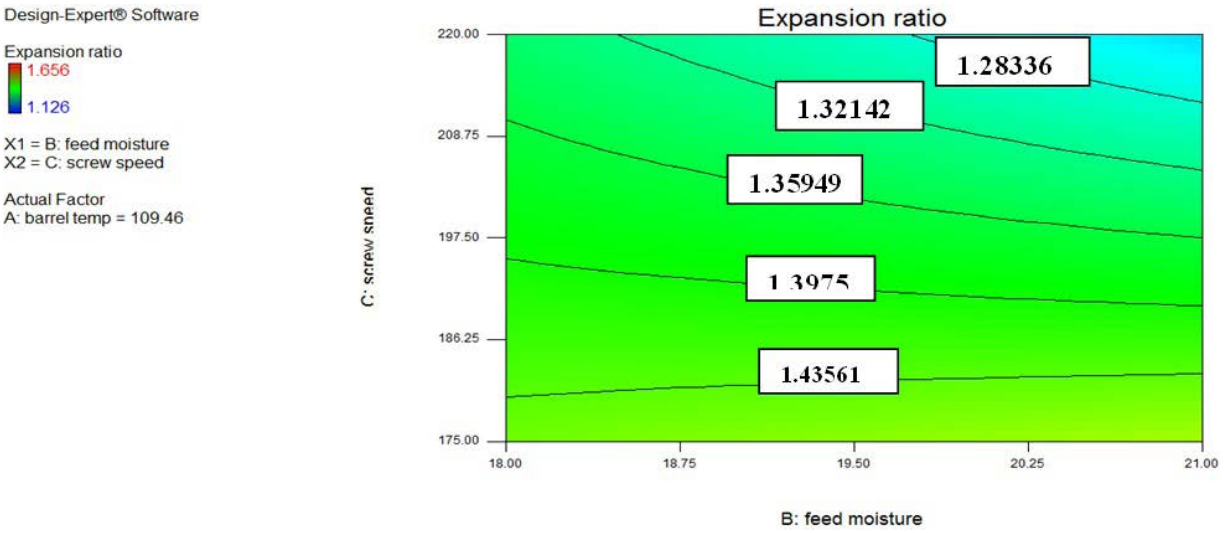
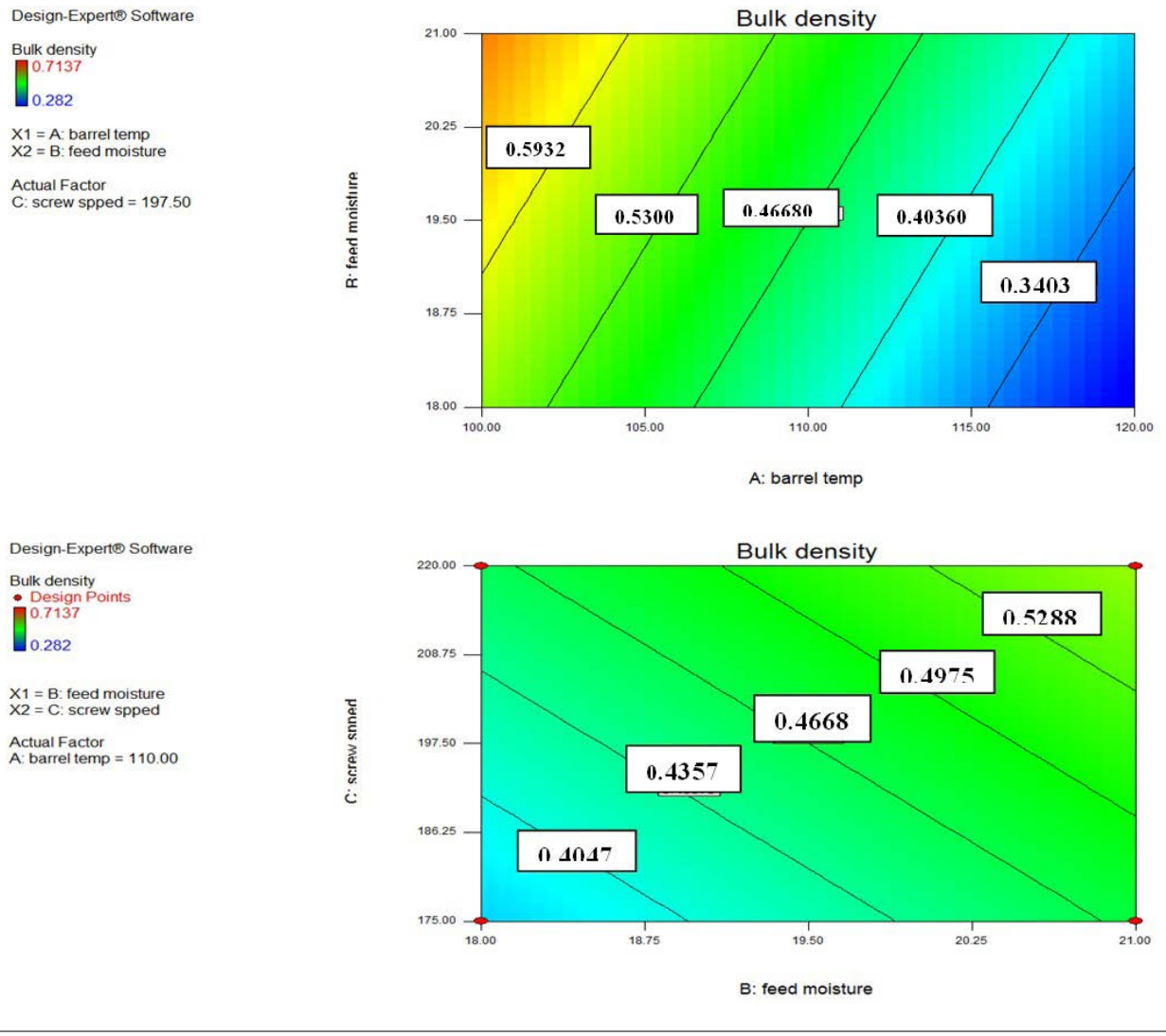


Figure 9. *Effect of Barrel Temperature, Feed Moisture and Screw Speed on the Bulk Density of Extrudate Contour Line*



ponent to form puff structure. The result shows that as screw speed and feed moisture increases the bulk density of extrudate also increases. However, bulk density values ranged from 0.282 to 0.7137 g/cm^3 and this shows that quality of extrudate was high at lower values of bulk density as decreases screw speed and feed moisture with increasing barrel temperature. But, barrel temperature increases beyond the maximum limit bulk density goes to decrease due to plasticization and causing a reduction in melting and gelatinization of dough. Evidence from cohort findings shows that feed moisture and barrel temperature are detriments of bulk density as well as expansion ratio. The result from these studies is a cohort and has similar ranges with other findings.[33,36]

Water absorption indices (WAI): WAI and WSI are very important characteristics of extrudate and measured the degree of starch damage or conversion into amylase and amylopectin due to the effect of extrusion processing parameter.[34] WAI measures the amount of water absorbed by starch component, which maintains the integrity of starch in aqueous dispersion,[16] and shows the index of starch gelatinization. The ANOVA shows that linear terms of screw speed and feed moisture affect WAI significantly (p<0.05).

Values of WAI range from 495.09 to 731.99% of the extrudate. Figure 10 shows that, feed moisture increased to result in increasing WAI. Higher feed moisture which is increased gelatinization of starch in continues heating that causes to increase in WAI of extrudate.[31] WAI also decreased as increasing of screw speed, which means that at a higher level of screw speed was make fragmentation of starch molecules and cause to lose the structure of starch in aqueous dispersion. But WAI increased at a higher temperature before plasticization occurs.[37]

Water solubility indices (WSI): The water solubility indices used as an indicator of degradation and conversion of starch molecules into water-soluble polysaccharides such as amylase and amylopectin.[38] The barrel temperature, feed moisture and screw speed of the extrusion parameter would affect the WSI of the extrudate, significantly p<0.05. Values of WSI range from 6.87-17.28% at constant screw speed which is presented in Figure 11. Decreasing of feed moisture and barrel temperature would cause to increase in WSI. This is due to that at lower moisture content, the viscosity of starch was high and enhance starch degradation but decrease starch gelatinization.[34] Similar effect was also reported in Ding et al, works.[31]

WSI also increases significantly p<0.05 as increasing screw speed, but decreasing in barrel temperature and feed moisture. Higher values of screw speed cause to develop high mixing energy at low feed moisture results to enhance starch degradation and fragmentation, which are more soluble in water. But at low screw speed with high feed moisture cause to develop low mixing energy results to enhance starch gelatinization and decrease degradation, which means an increase in WAI and decrease in WSI at constant heating barrel temperature.

Proximate Composition of Biscuit and Extrudate Sample

The proximate composition and gross energy of biscuit sample were presented in Table 4. Based on sensory analysis and fracture strength (crispness) of biscuit samples, we selected only three samples (B1, B3 and B4) analyzed their proximate composition, minerals and bioactive components from total seven samples including the control. The major difference between the three samples was mainly based on their crude protein. B3 had higher

Figure 10. *Effect of Barrel Temperature, Feed Moisture and Screw Speed on Wai of Extrudate*

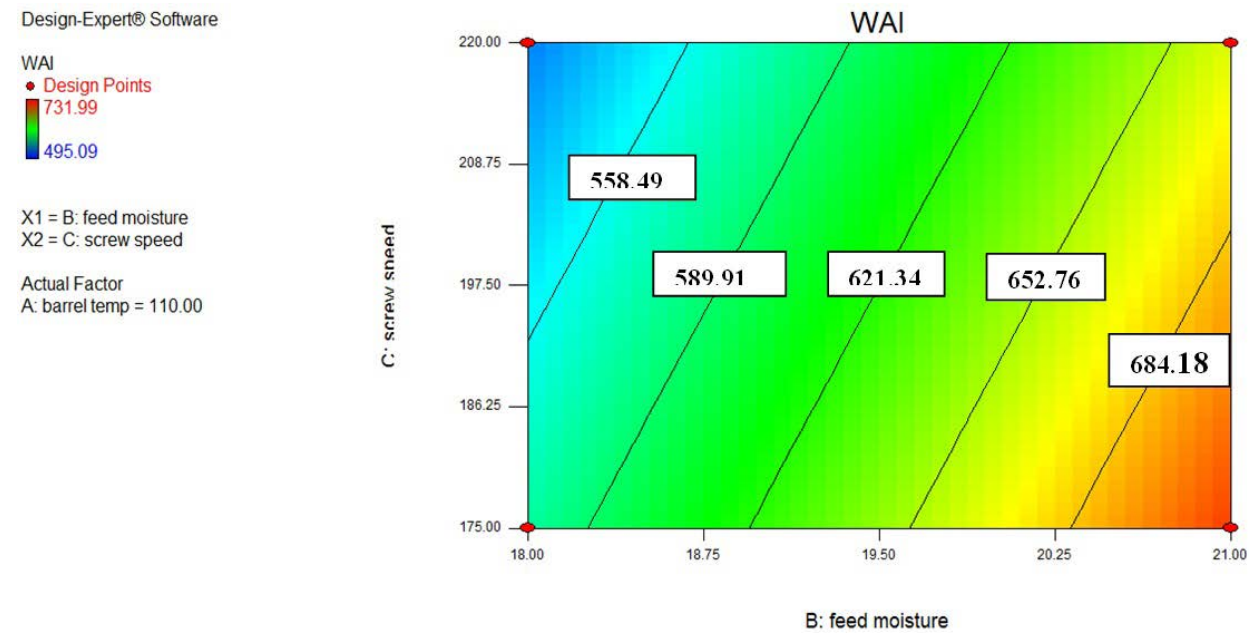
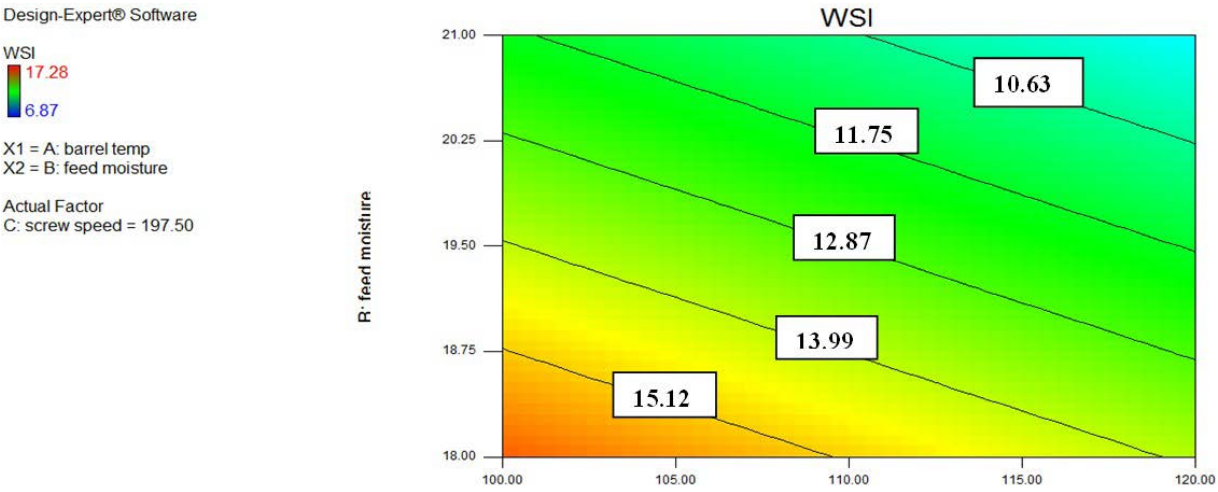
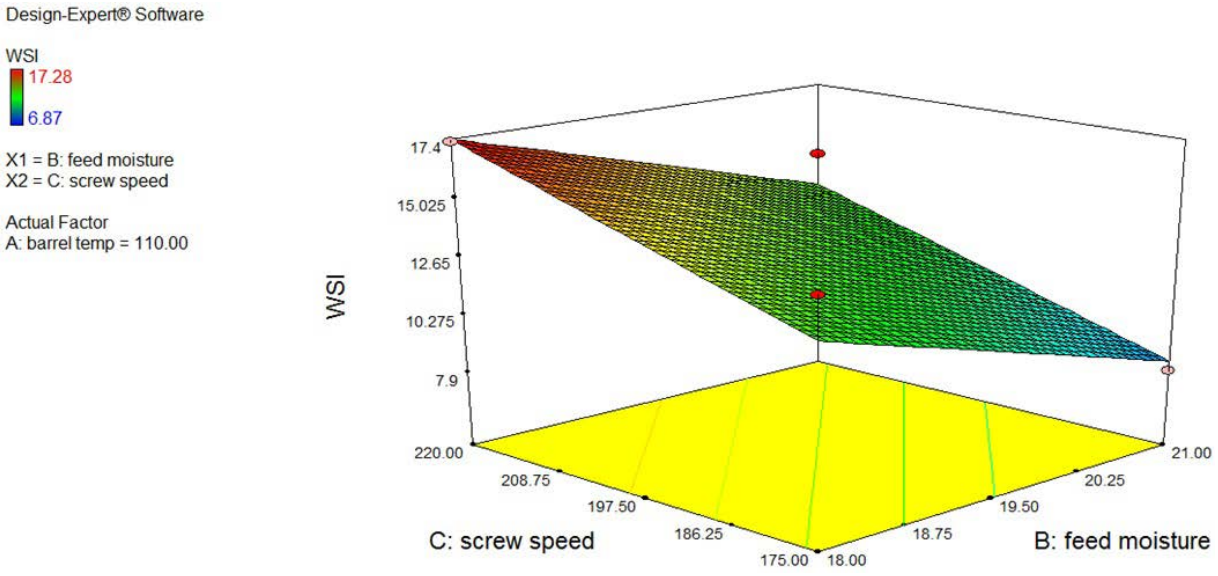


Figure 11. *Effect of Barrel Temperature, Feed Moisture and Screw Speed on Wsi of Extrudate*





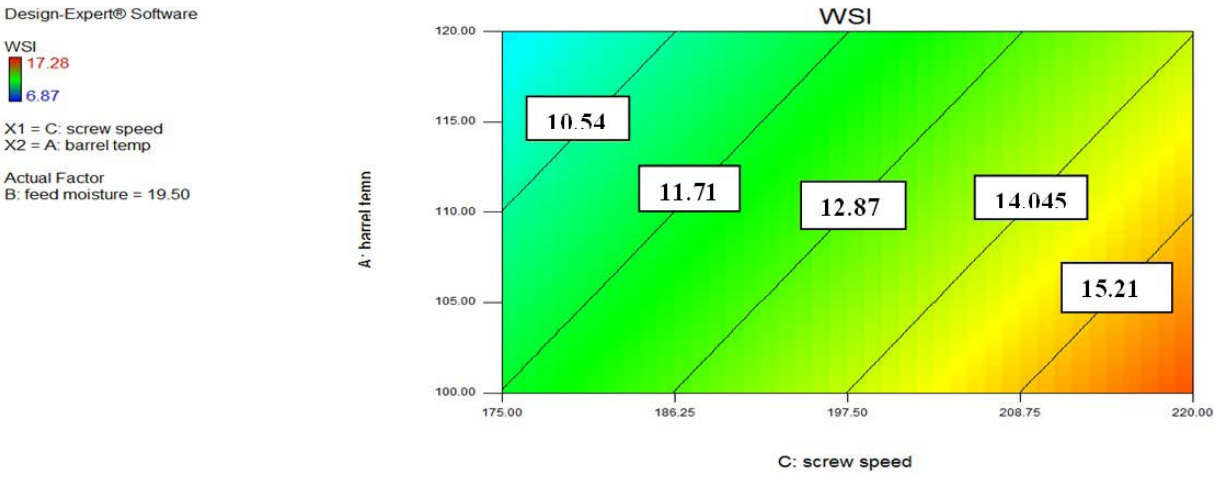


Table 4. *Proximate Composition and Energy of Biscuit Samples*

Code	Moisture (%)	Crude Fiber (%)	Fat (%)	Crude Protein (%)	Total Ash (%)	Carbohydrate (%)	Energy (kJ/100g)
B1	6.219±0.128[a]	2.105±0.063[b]	10.558±0.079[c]	12.803±0.089[c]	1.905±0.021[a]	66.381±0.05[a]	1738.515±2.936[c]
B3	5.392±0.006[c]	2.200±0.240[b]	11.632±0.075[b]	15.972±0.125[a]	1.930±0.084[a]	62.872±0.13[c]	1773.671±5.113[b]
B4	5.835±0.059[b]	2.655±0.318[a]	12.569±0.149[a]	14.211±0.143[b]	1.68±0.014[b]	63.048±0.39[b]	1786.336±7.010[a]

[a-c]All values are means of triplicate determination±SD and expressed in dry basis, except moisture

crude protein concentration than the other biscuit samples, this result shows that, it has a good protein and could influences costumer choose over the control biscuit sample. This result also coherent with other findings.[25] The total gross energy also higher in B4, B3 and B1 respectively, but the higher gross energy was recorded in B4 because of higher in carbohydrate and fat composition of biscuit sample raw material constituent. Crude fiber result also has low in B1 and higher in B4 corresponding to the wheat flour concentration in biscuit sample. The moisture of the biscuit sample also an important factor in determining storage condition and shelf life of biscuit sample and this result may good in order to preserve from microbial growth in addition to packaging materials.

The proximate composition and gross energy of extrudate also presented in Table 5. Depending on the sensory result, we were selected two extrudate among five extrudates and make a further physico-chemical analysis. Flake extrudate (Ex-3), has higher in protein concentration, carbohydrate, total energy, and total ash but lower in moisture, crude fiber and ash compared to Ex-4 which has lower cowpea during extrudate production. Extruded products are useful for children and adults based on their carbohydrate and protein sources beyond their minerals. The aims of this studies also produce such products which have higher in protein and carbohydrates as well as their energy values like Ex-3 beyond that how much cowpea flour substitute in the production of wheat-based products and preferred over extruded produce from wheat flour only. Similar findings in product development from cowpea and wheat flour show that increase in protein concentration due to incremental of cowpea flour for different types of Nigerian products according to Akubor (2003) and Akubor (2004).[25,39]

Mineral Composition of Biscuit and Extrude

Mineral composition of biscuit and extruded samples were presented in Table 6. Cowpea flour is good sources of minerals as a whole and de-hulled one.[40] Calcium concentration of biscuit sample was higher in B1, while magnesium, iron, and zinc were higher in B4. Biscuit sample B3 was significantly differencing in iron concentration from another biscuit sample which is a minimum concentration exist in B3. Magnesium concentration was higher in B3 next to biscuit sample B4. The iron and zinc mineral concentration of extrudate also higher in Ex-3 compared to Ex-4. Whereas, calcium and magnesium concentration was higher in B4 extruded samples.

Table 5. *Proximate Composition and Energy of Extruded Samples*

Code	Moisture (%)	Crude Fiber (%)	Fat (%)	Crude Protein (%)	Total Ash (%)	Carbohydrate (%)	Energy (kJ/100g)
Ex-3	6.35±0.028[b]	2.22±0.014[b]	0.67±0.042[b]	15.915±0.251[a]	2.505±0.035[a]	72.340±0.315[a]	1517.024±0.612[a]
Ex-4	10.6±0.014[a]	4.36±0.028[a]	0.775±0.021[a]	14.256±0.127[b]	2.185±0.036[b]	67.822±0.098[b]	1435.655±0.098[b]

[a-b]All values are means of triplicate determination±SD and expressed in dry basis, except moisture.
Where: B1 (90:10), B3 & (Ex-3) (65:35), and B4 & (Ex-4) (78:22) (wheat to cowpea flour), respectively.
Values expressed with similar subscripts are not significantly different by Duncan multiple range test across a column (p<0.05).

Table 6. *Mineral Composition of Biscuit and Extruded Samples*

Biscuit & Extrudate Sample	Ca (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)
B1	3.824±0.024[a]	2.564±0.002[c]	1.081±0.019[b]	1.505±0.028[ab]
B3	2.205±0.080[c]	2.571±0.003[b]	0.836±0.018[c]	1.386±0.045[b]
B4	2.849±0.027[b]	2.575±0.001[a]	1.369±0.002[a]	1.590±0.107[a]
Ex-3	6.601±0.603[b]	1.887±0.087[b]	1.661±0.606[a]	1.537±0.020[a]
Ex-4	6.976±0.520[a]	1.999±0.465[a]	0.546±0.089[b]	1.408±0.003[b]

[a-c]All values were means of duplicate determination±SD (Standard deviation).
Where: B1 (90:10), B3 & (Ex-3) (65:35), and B4 & (Ex-4) (78:22) (wheat, cowpea flour), respectively.
• Values expressed with similar subscripts are not significantly different by Duncan multiple range Test across the columns (p<0.05).
• Ex-3 and Ex-4-Extruded samples three and four

Table 7. *Tannin and Phytate Concentration of Biscuit and Extrudate Products*

	Biscuit Sample (mg/100g)			Extrudate Sample (mg/100g)	
s/code	**B1**	**B3**	**B4**	**Ex-3**	**Ex-4**
Tannin	69.723±6.061[a]	61.193±0.000[b]	32.540±0.000[c]	BDL	BDL
Phytate	70.301±17.42[e]	89.552±8.167[d]	108.528±0.945[c]	122.240±5.601[b]	125.28±2.984[a]

[a-e]All values were the means of duplicate determination±SD (Standard deviation).
Values expressed with similar subscripts are not significantly different by Duncan multiple range Test across the row ($p<0.05$).
Where: BDL-Below detection limit.

Table 8. *Microbial Load of Biscuit and Extruded Samples*

		Biscuit Samples			Extrudate Samples	
Types of Microbe	**Dilution Series**	**B1**	**B3**	**B4**	**Ex-3**	**Ex-4**
Aerobic bacteria, 35 °C	10^{-5}	37	42	225	42	46.5
Yeast & Molds 25 °C	10^{-3}	69	BDL	32	BDL	BDL

All results were triplicate determination. Where: BDL-Below detection limit.

The Bioactive Component in Biscuit and Extruded Samples

Bioactive component (tannin and phytate) analyses of products were presented in Table 7. The result of tannin analysis after processing reveled mg/100 g. Whereas, 69.723 mg/100 g was the highest values of tannin in B1, and 32.540 mg/100 g was the lowest value in B4 biscuit sample. Furthermore, the tannin in extruded sample was below the detection limit. Phytate concentration was highest in B4 and lowest in B1 biscuit sample and also higher in Ex-4 and lower in Ex-3 in Flake-extruded samples.

The evidence from epidemiological studies indicate that diets rich in pulse grains are also associated with a lower risk of several degenerative diseases, because of that pulse based products contain several non-nutritive bioactive compounds (health-promoting mixture) of phytochemical's which act as natural antioxidants and protects deoxyribonucleic acid (DNA) damage,[41] like phytate act as anticarcinogen and regulates chromatin remodeling, endocytosis, and nuclear messenger Image result for rNA full form www.javatpoint.com[42] ribonucleic acid (RNA) export. The phenolic compound which consists of tannin act as antioxidant properties due to their potential for oxidation and reduction process, which enables them to act as reducing agents, donating hydrogen and neutralizing free radicals.[43]

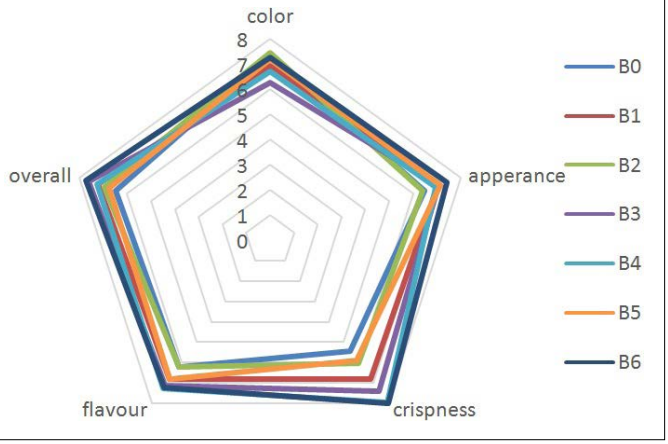
The Microbial Load of Biscuit Extruded Sample

Microbial load (total plate count, yeast and molds) of samples result were analyzed and presented in Table 8 before sensory analysis was performed. Biscuit and extruded sample generally have a lower aerobic-bacteria and yeast and mold at 10^{-5} and 10^{-3} series dilution respectively. Even, yeast and mold were not detected in biscuit B3 and extruded sample. This result also shows a smaller number of aerobic bacteria and yeast and mold counts of other coherent studies.[44] This result was in safe limits and it shows cowpea flour addition in product development do not have any microbial related problems.

Sensory Evaluation of Biscuit and Flake-extruded Samples

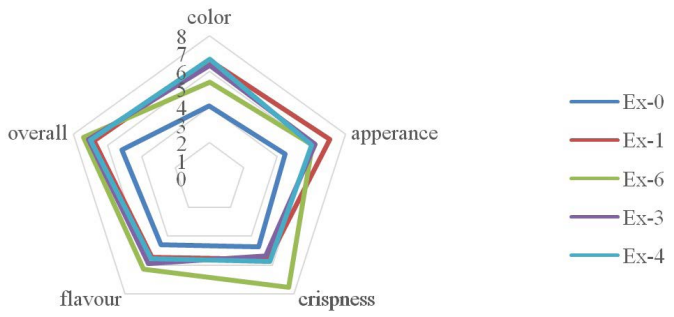
Each product was evaluated for color, appearance, crispness, flavor and overall acceptability with nine hedonic scales.[18] According to untrained and semi-trained panelist, the color evaluation was performed for the products and shows that B2 has a higher value and B3 has lower values. As shown in radar plots of biscuit samples sensory evaluation results shows as the cowpea flour concentration increases, the color acceptance of the product was a higher degree of acceptance (Figure 12). Nevertheless, in crispness, the higher degree of acceptance was depended upon the concentration of the wheat flour rather than the cowpea flour in the products. This is due to the content of gluten which is found in wheat and forms porous internal structure during cooking or baking and it helps to lose moisture easily. In B0, B2 and B5, flavor acceptance was lies between like slightly and like moderately, but in B1, B3, B4 and B6 (control) flavor was higher acceptance (like moderately). In general, the sensory result shows that B3 has higher overall acceptance next to B6 (control) and B0 has a lower overall acceptance.

Figure 12. *Radar Plots of Biscuit Sample Sensory Evaluation Results*



In flake-extrudate, Ex-4, Ex-1, and Ex-3 have a higher in color, appearance, crispiness, and flavor; respectively (Figure 13). The crispness of the extrudate was a higher degree of acceptance which is the wheat flour concentration in extruded was higher and the bole type cowpea flour was in low amount. The whole color was higher in Ex-1, Ex-4, and Ex-3 of extrudate which is prepared from the blending of the two flour, this higher acceptance may be due to the browning reaction of sugar (carbohydrate) of two flour dough and the higher concentration of protein in cowpea flour at a higher temperature. But, extruded produce in independent flour was a lower degree of acceptance of color. Ex-3 and Ex-4 have a value greater than 7 (like moderately) which is similar to Ex-6 (control) of the overall degree of acceptance.

Figure 13. *Radar Plots of an Extruded Product Sensory Evaluation Results*



Where: Ex-0 (0:100), Ex-1(90:10), Ex-6 (100:0), Ex-3(65:35), and Ex-4(78:22) (wheat to cowpea) flour, respectively.

CONCLUSION

Based on the results, it was revealed that cowpea-wheat blended flour have a good nutritional quality on the biscuit and extrudate products. Crude protein for biscuit and extruded products were increased as a result of increasing the amount of cowpea flour addition. Cowpea flour with 35% blending ratio could substitute in both biscuit and extruded products which in turn have best sensory quality in terms of crispness (low fracture strength). The best-fitted extrusion process parameter for the extrudate was 1200C barrel temperature, 21% feed moisture and 175 rpm screw speed. The extrusion parameters including expansion ratio, bulk density and WAI index were having a good quality measure of the extrudate product which was produced from 35% of cowpea and 65% wheat flour blends. Nevertheless, further investigation on product amino-acid profile, protein and starch digestibility and mineral bioavailability are required for commercial application of the products.

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COMPLIANCE WITH ETHICAL STANDARDS

This study protocol for sensory quality analysis was reviewed and approved by the institutional review board of the Addis Ababa Institute of Technology, School of Chemical and Bio-Engineering, Food Engineering Graduate Program. Informed consent was waived by the board. The manuscript is in line with the International recommendations for the ethical standards.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

Therapeutic Value of Garlic (*Allium sativum*): A Review

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ABSTRACT

Garlic (*Allium sativum*) is a source of medicine in many ways in human beings in routine life as well as in animals and its leaves, flowers, and cloves have been used in traditional medicine for a long time. Research in recent decades has shown widespread pharmacological and therapeutic effects of *A. sativum* and its organosulfur compounds especially allicin. The most important chemical constituents of this plant are organosulfur compounds such as allicin, diallyl disulphide, S-allylcysteine, and diallyl trisulfide. These chemicals were used for the treatment of inflammation, cancer, blood pressure, atherosclerosis, and hyperlipidemia as praised by several authors. Additionally, extracts of garlic have been used to treat various diseases and have shown anti-viral, anti-bacterial, anti-fungal, anticoagulative and antioxidant effects. However, few adverse effects have been found with garlic are nausea and vomiting when high quantity consumed. To review the therapeutic values of garlic and its importance in human and veterinary practices. Garlic is safe and rich sources of biologically active compounds with low toxicity. Further studies are needed to confirm the safety and quality of the plants to be used by clinicians as therapeutic agents.

Keywords
Allium sativum; Therapeutic values; Antibacterial; Antifungal; Antiviral; Anticancer; Anticoagulative; Antioxidant; Anti-inflammatory.

INTRODUCTION

Traditional medicines occupy a valuable place amongst rural groups of developing countries for the provision of health care inside the absence of an efficient public health care scheme.[1] The use of traditional treatments is common in sub-Saharan Africa, and visits to traditional healers remain a prime live of care for many people because of preference, affordability, limitation of practitioners and modern hospitals.[2] Moreover, traditional medicines may be the supply of remedy for lots of health complications.[3,4]

Garlic, *Allium sativum* is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular medicine for various diseases and physiological disorders. The word garlic was originated from the Celtic word meaning pungent. Garlic is cultivated practically throughout the world and appears to have originated in central Asia and then spread to China, the Near East, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa (Egypt) and Mexico.[5-7] Garlic has played an essential role for over 7,000-years in central Asia, Africa, Europe, and the Mediterranean region.[8]

Garlic is a bulb growing to 25-70 cm with hermaphrodite flowers where its leaves and cloves have been used in traditional medicine for a long time. Aged garlic is used to make aged garlic extract (AGE), a popular herbal supplement that has been proven to boost the immune system and possibly prevent cancer and cardiovascular disease. Additionally, as garlic ages, it loses its strong flavor, so there is no need to worry about breath odor. Garlic can be used for culinary and medicinal purposes. The culinary use includes spicy flavor that mellows and sweetens considerably with cooking. While its medicinal uses include treatment of whooping cough, lung disease, stomach complaint and disorder resulting from childbirth, cold, sore eyes, and earache as well as help in the prevention of heart disease.[3,9,10] A study on Czech revealed that garlic oil especially dehydrated powder could help in reducing the accumulation of cholesterol in the vascular walls of animals.[11,12]

Allicin (diallyl-dithiosulfinate) is the most important component of garlic and generally claimed to be responsible for its numerous beneficial effects including antibacterial, antiviral, anti-

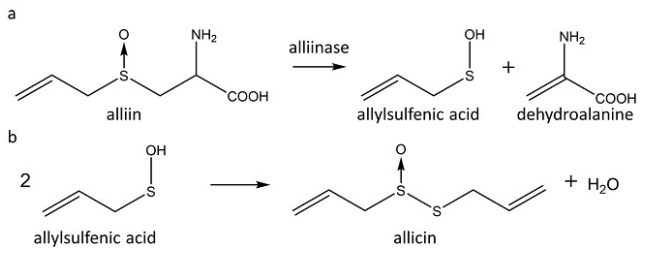
fungal and antioxidative effect. Nowadays, the alarming growth of the number of antibiotic-resistant bacteria and difficulties in the treatment of infections has initiated a search for new antibacterial compounds and develop new alternative strategies in combating bacterial infections. Medicinal plants such as garlic, with their long history of use in folk medicine for the treatment of infectious diseases, have become a promising new source of antibacterial agents. Besides, they exhibit a direct antimicrobial activity and/or an indirect activity through synergism with antibiotics that increase their effectiveness.[13] Hence, this review was prepared with the aim of increasing awareness on the medicinal importance of *A. sativum* in human and veterinary medicine

ALLIUM SATIVUM

Biosynthesis

Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in aqueous garlic extract or raw garlic homogenate. It is produced from the non-proteinogenic acid alliin (S-allyl cysteine sulfoxide) upon tissue damage in a reaction that is catalyzed by the enzyme alliinase (Figure 1). Alliinase enzyme is activated when garlic is chopped or crushed and acts on alliin (present in raw garlic) to produce allicin. In addition, structurally analogous thiosulfinates are produced in nature by other *Allium* species.[4,14]

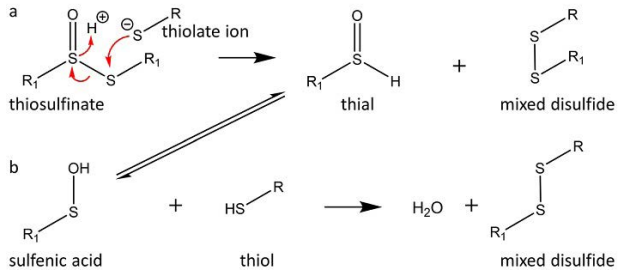
Figure 1. *Biosynthesis of Allicin from S-allyl Cysteine Sulfoxide (alliin) (a). The Enzyme Alliinase Catalyzes the Formation of Allyl Sulfenic Acid and Dehydroalanine, (b). Whereupon Two Molecules of Allyl Sulfenic Acid Condense Spontaneously to Yield One Molecule of Allicin.*



Adapted from Leontiev R[15]

Allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate, and γ-L-glutamyl-S-alkyl-L-cysteine are other important sulfur-containing compounds present in garlic. The thiosulfinates are condensation products of two sulfenic acids to form disulfide-S-monoxides and can be viewed as 'sulfenic acid anhydrides. In the laboratory, allicin can be synthesized most effectively by oxidation of diallyl disulfide (DADS) with H_2O_2 in the presence of an organic acid catalyst that is first oxidized to the corresponding peroxy-acid, like performic acid or peracetic acid.[16] The reactivity of thiosulfinates towards thiol-groups is an important component of their antimicrobial activity. The electron-withdrawing effect of the O-atom creates an electrophilic sulfur center which reacts readily with thiols, or more specifically, with thiolate ions (Figure 2), thereby forming an S-allylmercapto adduct.[17]

Figure 2. *Reaction Mechanism of a Thiol with a Thiosulfinate. (a). The Reaction Leads Directly, (b) Indirectly, to the Formation of a Mixed Disulfide, which Under Some Conditions may React Further with RSH in a Thiol-disulfide Exchange Reaction to form RSSR and R_1SH.*



Adapted from Leontiev R[15]

Chemical Constituents of *Allium sativum* and its Nutritional Value

A. sativum contains about 33 sulfur compounds, several enzymes,[17] amino acids, and minerals such as selenium. Studies carried out on the chemical composition of the garlic show that sulfur compounds such as allicin are important constituents of the plant that is responsible for many of its medicinal effects and garlic's pungent odor. Besides, diallyl disulphide (DDS), S-allylcysteine (SAC) and diallyl trisulfide (DTS) are other sulfur compounds that have some roles in the therapeutic effects of the plant. DTS is a chemically stable final transformation product of allicin.[3,18]

Garlic powder is a simply dehydrated and crushed garlic clove. Besides, the water extract of heat-treated garlic contains mainly alliin. Accordingly, enzymatic activity of alliinase of garlic powder is similar to that of fresh garlic. However, dehydration temperature should not exceed 60 °C, above which alliinase is inactivated.[3,19] In contrast to fresh garlic and garlic powder, Garlic oil, and steam-distilled garlic do not have significant amounts of alliin or allicin, but instead, contain various products of allicin transformation. Garlic has been analyzed for moisture, carbohydrate, protein, fat, minerals, vitamins, energy, ash, pH, acidity and essential oil contents.[20,21] Some of the nutritional and chemical properties of garlic bulbs are summarized in Table 1.

Table 1. *Nutritional Value and Properties of Allium Sativum; Values Expressed per 100 g of Raw Garlic (Allium sativum)*

Properties	Values	Minerals	Values	Vitamins	Values
Energy	119 Kcal	Potassium	446 mg	Thiamine	0.16 mg
Moisture	70%	Phosphorus	134 mg	Riboflavin	0.02 mg
Protein	4.3 g	Magnesium	24.1 mg	Niacin	1.02 mg
Carbohydrate	24.3 g	Sodium	19 mg	Pyridoxine	0.32 mg
Fiber	1.2 g	Calcium	17.8 mg	Folic acid	4.8 µg
Fat	0.23 g	Iron	1.2 mg	Ascorbic acid	14 mg
Alcohol	0 g	Zinc	1.1 mg	Carotenoids	5 µg
Ash	2.3%	Iodine	4.7 µg	Vitamin A	Traces
PH	6.05	Selenium	2 µg	Vitamin E	0.011 µg
Acidity	0.172%				

Source: Pacurar et al[22]

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Historical Background and Traditional uses of *Allium Sativum*

The ancient Egyptians used garlic to treat diarrhea and its potential medical value was described on the walls of ancient temples and on papyrus dating to 1500 BC. It was used by Greek physicians Hippocrates and Galen to treat intestinal and extra-intestinal diseases; ancient Japanese and Chinese used it to treat headaches, flu, sore throat, and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media, and respiratory tract infections.[6,8]

Garlic is nicknamed as Russian penicillin due to its widespread use as a topical and systemic antimicrobial agent; it is commonly used in many cultures as an excitement and reputation of healing power. Garlic was used to treat common colds, hay fever and asthma in Europe and India.[23] Leaves and cloves of *A. sativum* have been used in traditional medicine of Iran and other countries for a long time.[24,25] In the pre-antibiotic era, allicin can kill bacteria *via* the gas phase and was used to successfully treat many lung-pathogenic bacteria such as tuberculosis from crushed garlic preparations through breathing in the vapor.[26]

MEDICINAL IMPORTANCE OF ALLIUM SATIVUM

Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. Antifungal, antiviral, antibacterial, anthelmintic, antiseptic and anti-inflammatory properties of garlic have been well documented. The extracts exhibited a pronounced activity against both gram-negative (*E. coli*, Salmonella species, and Citrobacter Enterobacter, *Pseudomonas klebsiella*) and gram-positive (*Staphylococcus aureus, S. pneumonia* Group A *streptococcus,* and *Bacillus anthrax*) bacteria causing considerable morbidity worldwide.[4,6,27]

Antimicrobial Activity

Allicin and other sulfur compounds are thought to be the major compounds responsible for the antimicrobial effect of garlic. The antimicrobial properties of garlic were first described by Pasteur in 1958, and since then, many researches had demonstrated its effectiveness and broad-spectrum antimicrobial activity against many species of bacteria, viruses, parasites, protozoan and fungi.[26,28] Garlic is more effective with the least side effects as compared to commercial antibiotics; as a result, they are used as an alternative remedy for the treatment of various infections.[29,30] Out of the many medicinal plants, garlic has an antimicrobial property that protects the host from other pathogens highlighting the importance of search for natural antimicrobial drugs.[28,31] Previously conducted researches confirmed that garlic is not only effective against Gram-positive and Gram-negative bacteria but also possesses antiviral and antifungal activities.[26,32,33]

Anti-bacterial activity: Garlic has been used for centuries in various societies to combat infectious disease. According to different research findings, garlic has been proven to be effective against a plethora of gram-positive, gram-negative, and acid-fast bacteria. These include Salmonella, *Escherichia coli*. Pseudomonas, Proteus, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtulis*, Salmonella spp., Klebsiella spp., Micrococcus spp., Clostridium spp. and Mycobacterium spp.[28,31,33,34]

The gram-positive *Staphylococcus aureus* was more susceptible to the toxic effects of garlic than its gram-negative counterparts. It has been shown that Gram-negative diarrheagenic pathogens (*E. coli*, *Proteus mirabilis*, Shigella spp., and Salmonella spp.,) from stool samples were highly sensitive to garlic.[34] It has been shown that the aqueous extract of garlic can be used alongside conventional antibiotics to fight agents of nosocomial infections in hospitals.[24] *In vitro* and *in vivo* study of garlic extract was also effective against *Streptococcus mutans* which is primary etiological organisms in dental caries.[5,9,34]

The cloves of garlic and rhizomes of ginger, extracted with 95% ethanol, suggested to have antibacterial activity against multi-drug clinical pathogens and can be used for prevention of drug resistant microbial diseases. *Pseudomonas aeruginosa* was the most sensitive germ to the mixture. Garlic also suggested as a treatment for multi-drug resistant tuberculosis. Besides. allicin in its pure form was found to exhibit antibacterial activity against multidrug-resistant enterotoxicogenic strains of *E. coli*.[33,35] In a study by Lai and Roy, fresh extracts of *A. sativum* (garlic) and *Nigella sativum* (black cumin) had more antibacterial activity against the isolates of the urinary tract infection than cefalexin, cotrimoxazole, and nalidixic acid. Garlic, allyl methyl sulfide, has antibacterial activity against the pig pathogen *Actinobacillus pleuropneumoniae* serotype 9.[9,24]

Anti-viral activity: Garlic and its sulfur constituents verified antiviral activity against Coxsackievirus species, Herpes simplex virus types 1 and 2, Influenza B, Para-influenza virus type 3, Vaccinia virus, Vesicular stomatitis virus, Human immunodeficiency virus type 1 and Human rhinovirus type 2. The order of compounds found in garlic for virucidal activity was, ajoene>allicin>allyl methyl thiosulfate>methyl allyl thiosulfate; no activity was found for the polar fractions, alliin, deoxy alliin, diallyl disulfide, or diallyl trisulfide. According to different research findings, garlic is an effectual treatment for both the influenza B virus and herpes simplex virus. Two independent researchers in Japan and Romania have found that garlic is able to protect living organisms from the influenza virus and enhanced the production of neutralizing antibodies when given the vaccine.[24,36,37]

Ajoene, isolated from extracts of garlic may inhibit adhesive interaction and fusion of leukocytes. In a study investigating the effect of allitridin (diallyl trisulfide) on the replication of human cytomegalovirus (HCMV) and the expression of viral immediate-early genes, it was revealed that this substance has anti-HCMV efficacy.[38] In another study, it was supposed that the antiviral activity of garlic in humans may be secondary to a direct toxic effect on viruses. It also enhanced the NK-cell (Natural killer-cell) activity that destroys virus-infected cells.[24] On, a double-blind placebo-controlled study has shown significant protection from the common cold virus and used for prevention, treatment and reduction of reinfection benefits from taking allimax powder capsules once daily.[4,11,39]

Anti-fungal activity: Different dilutions of extracts of *A. sativum* have been shown to possess fungistatic and fungicidal activity *in vitro* and *in vivo*. Ajoene is an active compound found in garlic which plays a great role as a topical antifungal agent. Garlic has been shown to inhibit the growth of fungal diseases as equally as the drug ketoconazole, when tested on the fungi *Malassezia furfur*, *Candida albicans*, *Aspergillus*, Cryptococcus and other Candida species.[11,40] A report from a Chinese medical journal delineates the use of intravenous garlic to treat a potentially fatal fungal brain infection called *Cryptococcus meningitis*. Studies on the effect of amphotericin B (AmB) against Candida. albicans showed that allicin enhances significantly the effect of AmB against *Candida albicans*, *Saccharomyces cerevisiae* and against *Aspergillus fumigatus in vitro* and *in vivo*.[24,40]

An *in vivo* study showed that antibody-alliinase conjugates and alliin are effective against murine pulmonary aspergillosis.[41] An *in vitro* study showed both intrinsic antifungal activity of allicin and its synergy with the azoles group of drugs, in the treatment of candidiasis.[42] One study showed that six different mixtures of garlic distilled oils containing DDS and DTS are active against a number of yeasts (*C. albicans*, *C. tropicalis*, and *Blastoschizomyces capitatus*).[30] Saponins from *A. sativum* were shown to be effective against *Botrytis cinerea* and *Trichoderma harzianum*.[43] Another antifungal protein, allivin, was isolated from *A. sativum* with antifungal activity against *Botrytis cinerea*, *Mycosphaerella arachidicola* and *Physalospor apiricola*.[24]

According to the study made in mice, liquid garlic extract was having a substantial effect in reducing the Candida colonies in mice through stimulating the body's own defenses to enhance the phagocytic activity of the cells. Garlic oil can be used to treat ringworm, skin parasites and warts if it is applied externally. Lesions that were caused by skin fungi in rabbits and guinea pigs were treated with external applications of garlic extract and began to heal after seven days.[4,24]

Anti-Cancer Activity

Among the most prominent and favorable effects of garlic is its effect on the inhibition of the growth of cancer cells. Diallyl trisulphide (DATS) is one of the components of garlic that has a great effect on fighting cancer cells. The cytotoxicity caused by DATS is mediated by the generation of reactive oxygen species (ROS) and subsequent activation of the ROS-dependent caspase pathway in U937 leukemia cells. The action of garlic has been attributed to stimulating immune effector cells including T-cell and natural killer cells.[21,44] Numerous epidemiological, clinical and laboratory studies have demonstrated that garlic has a great role in cancer prevention especially in relation to digestive tract cancers. Different studies on humans have shown that regular intake of garlic reduces the risk of esophageal, stomach and colon cancer. This was thought to be due to the antioxidant effect of allicin in reducing the formation of carcinogenic compounds in the gastrointestinal tract.[27,45]

Garlic has also a variety of anti-tumor effects, including tumor cell growth inhibition and chemopreventive effects. In rodents, garlic and its constituents have been reported to inhibit the development of chemically induced tumors in the liver, colon prostate, bladder, mammary gland, esophagus, lung, skin, and stomach in both rodent and human studies.[36,46,47] DATS an organosulfur compound isolated from garlic has been shown anticancer activity both in *in vitro* and *in vivo* investigations. The cytotoxicity of DATS toward prostate epithelial cells reduced as opposed to PC-3 cancer cells.[48]

The toxic effect of garlic indirectly plays an important role in the death of cancer cells. Another key role in the prevention of cancers is garlic's effect on the immune system. Macrophage activity, NK as well as the cytokine tumor necrosis factor (TNF), were all shown to have increased activity after administration of garlic and this resulted in an increase in antitumor response.[4] Colorectal cancer is the third leading cause of cancer death in the world. In this respect, normal garlic cannot be administrated and would need to be introduced as part of a strict diet. The garlic and low meat diet, however, show a decrease in colorectal tumor growth.[6,11]

According to different research findings, aged garlic extract such as S-allyl cysteine, and S-allylmercapto-L-cysteine exhibited radical scavenging activity. In addition, S-allyl cysteine and some organosulfur compounds derived from garlic have been found to retard the growth of chemically induced and transplantable tumors in several animal models. Therefore, the consumption of garlic may provide some kind of protection from cancer development.[6,24]

Anti-Helminthic Activity

Development of anthelmintic resistance in helminths reported in a number of countries gives a clear indication that control programs based exclusively on their use are not sustainable. The development of integrated programs to control helminths is vital, but such control programs require viable alternatives to the use of anthelmintic.[49,50] Medicinal plants such as garlic have been used to treat parasitic infections in man and animals. A study showed that allicin is able to produce morphological changes in the male *Schistosoma mansoni*.[51] The alcoholic extract of bulb of *A. sativum* has also shown moderate *in vitro* anthelmintic activity against human *Ascaris lumbricoldes*. Garlic has been reported to be effective in the exposure of dysentery and possess anthelmintic activity against *Entamoeba histolytica* and *Giardia lamblia*.[4,52]

Diallyl trisulfide has *in vitro* activity against several important protozoan parasites. The results indicated that the compound has the potential to be used in the treatment of several human and animal parasitic diseases such as *Trypanosoma species*, *Entamoeba histolytica* and *Giardia lamblia*.[11,53,54] Garlic oil is effective against a wide range of protozoan parasites including *Plasmodium species*, *Trypanosoma species*, *Leishmania species*, *Giardia species*, and *Cochlospermum planchonii*. Its aqueous extract has also been shown to be effective against hymenolepiasis and giardiasis. In an *in vitro* study, the extracts of *A. sativum* were shown to have anthelmintic

activity against *Haemonchus contortus* from sheep by decreasing larval count. The ethanol extract was the most effective in decreasing larval count. In another study, aqueous extract from garlic has good activity against nematodes such as *Trichuris muris* and *Angiostrongylus cantonensis* when followed by chloroform extract.[6,22,55]

Garlic with a mixture of the different extracts was tested *in vivo* and *in vitro* for its anthelmintic activity against cestodes (*Hymenolepis diminuta*, *H. microstoma*, and *Taenia taeniaeformis*) and trematodes (*Fasciola hepatica*, *Echinostoma caproni*). In all *in vitro* tests, the target parasites died. In addition, the same composition was effective only against *Echino. Caproni* (intestinal fluke), while both worms were killed *in vitro*. The essential oil of *A. sativum* has a paralytic effect on *F. gigantica*.[56] The extract of *A. sativum* also possesses mosquito larvicidal properties. It is effective against filarial mosquito *Culexquinque fasciatus* (24-hour post-treatment), *Culexquinque fasciatus* and *Anopheles stephensi*.[7,57] Essential oil from *A. sativum* has acaricidal activity against *Rhipicephalus* (*Boophilus*) Microplus (*Canestrini*) tick larvae.[58] *A. sativum* has also been an insecticidal activity against larvae of *Aedesalbopictus* (*Skuse*), *Lycoriella ingénue*, and *Spodoptera litura* at 1000 ppm.[59,60]

Anti-Inflammatory Activity

Garlic extracts have been shown to exert anti-inflammatory effects.[61] In one study, garlic treatment significantly attenuated inflammation and injury of the liver induced by *Eimeria papillata* infections.[53] The anti-inflammatory activity exhibited by garlic oil is mainly through inhibiting the assembly-disassembly processes of the cytoskeleton.[62]

Cytokines involved in inflammatory bowel disease (IBD) direct a predominantly cell-mediated T-helper-1 (Th1) immune response. Several compounds isolated from *A. sativum* modulate leukocyte cell proliferation and cytokine production. To investigate the possible therapeutic effects of garlic in the treatment of patients with IBD, whole blood and peripheral blood mononuclear cells (PBMCs) should be assessed. In the presence of various concentrations of garlic extract, *in vitro*, the effect of garlic extract on leukocyte cytokine production was determined using multiparameter flow cytometry. Accordingly, inflammation associated with IBD can be treated with garlic extract by inhibiting Th1 and inflammatory cytokines while upregulating IL-10 production. An *in vivo* animal model study needs to be undertaken to determine the significance of these *in vitro* findings.[4,62,63]

Other authors have shown the preventive effect and possible toxicity of garlic oil and its organosulfur compounds in endotoxin-induced systemic inflammation and intestinal damage.[64] A lead compound derived from allicin is shown to be a good starting point for the development of anti-inflammatory drugs with fewer side effects.[65]

Anti-Oxidative Activity

Whole garlic knob and aged garlic extract exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalase, and glutathione peroxidase.[18] Garlic extract, allicin is efficiently scavenged exogenously generated hydroxyl radicals in a dose-dependent fashion, but their effectiveness was reduced by about 10% by heating to 100 °C for 20 min. The sulfur compounds such as S-allyl cysteine, found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude, aged garlic extract. Garlic (both the homogenate of 10% in physiological saline solution and its supernatant) was able to reduce the radicals present in cigarette smoke.[66]

In vivo, antioxidant effects of several garlic organosulfur compounds have been studied. In one study, two lipophilic organosulfur compounds, diallyl sulfide (DAS) and diallyl disulfide (DADS) and two hydrophilic organosulfur compounds, s-ethyl cysteine (SEC) and n-acetylcysteine (NAC), protected against lipid-related oxidations by activating associated antioxidant enzymes. The *in vivo* antioxidant effects of four test organosulfur compounds against lipid-associated oxidations have been studied by the researcher reported that these antioxidant effects were due to the activation and modification of several enzymes such as 3-hydroxy-3 methylglutaryl-CoA reductase, glutathione-s-transferase and catalase.[11,67]

Anti-Coagulant/Fibrinolytic Activity

Garlic and other species in the genus allium have played an important role as a prophylactic and therapeutic agent over centuries. Of these, the usefulness of garlic in preventing disease of the cardiovascular system is widely recognized. There are several reports on anticoagulants.[68] In a study, blood anticoagulant substance was isolated from garlic and its physical and chemical properties were also studied. A half milligram of garlic extracts completely inhibited one millitter of blood from coagulating. The inhibiting effect of garlic extract on blood clotting was almost the same as that of potassium oxalate.[3,4,11]

MECHANISMS OF ACTION AND SYNERGISTIC EFFECT OF ALLIUM SATIVUM

It is widely accepted that plant extracts, because of complex nature, possess multiple mechanisms of action. Garlic extracts and their main components may exhibit activity by: (i) inhibiting bacterial growth or viability, (ii) targeting bacterial virulence factors or (iii) potentiating the effectiveness of antibiotics as resistance modifying agents. The inhibition of bacterial growth occurs through several mechanisms: disruption of membrane function and structure (including the efflux system), interruption of deoxyribonucleic acid/ribonucleic acid (DNA/RNA) synthesis and function, interference with intermediary metabolism and induction of coagulation of cytoplasmic constituents (Table 2).[13,69,70]

Synergistic interaction between two agents, in which one agent enhances the effect of the other and together they act more efficiently than as individual agents. The mechanism of synergistic action is explained by (a) modification of active sites on bacterial cell, (b) inhibition of enzymes, which catalyze degradation or modification of antibiotics, (c) increase of membrane permeabil-

Table 2. *Mechanism of Action and Pharmacological Effects of Allium Sativum*

Effect	Pathogen	MOA	Preparation
Antibacterial	Staphylococcus aureus	Inhibition of bacteria growth	Aqueous, ethanol, chloroform extract
	Escherichia coli, Salmonella typhi	Higher inhibitory effect with Ethanolic extract and potentiating antibiotics effect	Aqueous and ethanolic extract
	Bacillus subtilis, Klebsiella pneumonia	Inhibition of bacteria growth with Ethanolic extract	Aqueous, methanol and ethanol extract
	Helicobacter pylori	Higher inhibitory effect	Extract
	Salmonella enteritidis		Extract
	Shigella spp, Proteus mirabilis		Extract
	Actinobacillus pleuropneumonia serotype 9	Inhibition of bacteria growth	Extract
	Streptococcus mutan		Extract
Antiviral	Human cytomegalovirus, Influenza B, Herpes simplex virus type 1-2, Parainfluenza virus type 3, vaccine virus, Vesicular stomatitis virus, Human rhinovirus type 2	Boost antibody production	Not mentioned
Antifungal	Candidia albicans, C. tropicalis, Blastoschizomyces capitatus	Inhibition by Changing antioxidant metabolites	Extract, DADS
	Botrytis cinerea, Trichoderma harzianum	Inhibition of fungal growth	Extract
	Ascosphaera apis		Essential oil vapors
	Paracoccidioides brasiliensis		Extract
	Aspergillus niger	Potentiating antibiotics effect	Extract, Ajoene
	Dermatophytes, Saprophytes, Candidia	Blockage of lipid synthesis	Ethanol extract
	Cryptococcal spp., Botr. cinerea, Mycosphaerella arachidicola, Physalospar apiricola	Inhibition of fungal growth	Alcoholic extract
Antiparasitic	Trypanosoma spp, Entamoeba hirtolytica, Giardia lamblia	Inhibition of glutathione reductase	Extract
	Schistosoma mansoni	Enhances morphological changes	Extract
	Trypanosoma cruzi T. brucei, Plasmodium spp, Giardia spp,	Inhibition of Trypanothione reductase.	Extract
	Leishmania spp, Cochlospermum planchomi	Inhibition of glutathione reductase	Extract
	Hymenolepiasis, Giardiasis	Muscular paralysis of the parasite	Aqueous extract
	Haemonchus contortus	Muscular paralysis of the parasite	Ethanol, dichloromethane and water extract
Other	Anti-inflammatory effect	Inhibition of assembly-disassembly processes of the cytoskeleton	Extract
	Antioxidant properties	Protection against free radical damage in the body	Organosulfur compound in garlic
	Anti-coagulant/ antithrombotic effect	Suppresses the coagulation system and inhibition of platelet aggregation	Extract
	Anti-tumor/cancer	Enhances immune effector cells, growth inhibition, and chemopreventive effects	Extract

Source: Mikaili et al[24]

ity and (d) inhibition of efflux pumps.[71]

Allicin, an antibacterial compound from garlic (*A. sativum*), potentiated the action of cefazolin (4 to 128-fold) and oxacillin (32 to 64-fold), against *Staphylococcus* spp. and cefoperazone (8 to 16-fold) against *P. aeruginosa*.[72] The significant antibacterial activity of garlic extract on streptomycin-resistant strains (*Staph. aureus* and *E. coli*) solely and in synergism with streptomycin has also been proved.[73] It was found in another study that polymyxin B (PMB), is effective against various yeasts and filamentous fungi when used in combination with allicin. This combination increases the plasma membrane permeability in *Saccharomyces cerevisiae*. The synergistic activity between PMB and allicin combinations resulted in the disappearance of the swollen spherical structure of the yeast as a result of structural alterations of its vacuole.[74] In addition, the synergism between ciprofloxacin and garlic extract has antibacterial activity against multi-drug clinical pathogens such as enterotoxicogenic strains of *E. coli* and mycobacterium.[29,75]

DRUG INTERACTION AND PHARMACOKINETICS OF GARLIC

Glutathione is a compound necessary for the liver to facilitate the detoxification of substances. Organo-sulfur compounds found in garlic showed to prevent glutathione depletion. Patients who experience increases in reactive oxygen-induced stress on liver function may be protected by garlic ingestion.[67] It was found in *E. coli* cultures that aged garlic extract, S-allyl cysteine, diallyl sulfide, and diallyl disulfide do not interfere with the antibiotic activity of gentamycin but may improve gentamycin-induced nephrotoxicity.[23] Aged garlic has also been shown to reverse the oxidant effects of nicotine toxicity in rat studies. More researches are required in future garlic may be a unique choice to help minimize

the toxic effects of therapeutic drugs.[3,8]

One study indicated that those who use traditional/complementary/alternative medicines (TCAMs) in addition to antiretroviral (ARV) treatment may be at risk of experiencing clinically significant pharmacokinetic interactions, particularly between the traditional complementary alternative medicines and the protease inhibitors as well as non-nucleoside reverse transcriptase inhibitors (NNRTIs). Mechanisms of pharmacokinetic interactions include alterations to the normal functioning of drug efflux transporters, such as P-gp and/or Cytochromes P450 (CYP) isoenzymes, such a CYP3A4 that mediates the absorption and elimination of drugs in the small intestine and liver. Specific mechanisms of action include inhibition and activation of these proteins and induction *via* the pregnane X receptor also known as the steroid and xenobiotic sensing nuclear receptor (SXR). Garlic exhibited potentially significant interactions, each with protease inhibitors or non-nucleoside reverse transcriptase inhibitors.[24,37]

In vivo absorption changes are possible between aged garlic extract and cardiovascular, antidiabetic and antiviral drugs, but the magnitude of the changes depends on the most profound process involved (influx, efflux, passive diffusion) in compound's permeability.[76] In a study, there was some pharmacokinetic interaction of garlic and atorvastatin in dyslipidemic rats was shown. It has been also shown that herbs such as garlic with the potential to significantly modulate the activity of drug-metabolizing enzymes (notably cytochrome P450 isozymes) and/or the drug transporter P-glycoprotein participate in potential pharmacokinetic interactions with anticancer drugs.[8,37]

ADVERSE EFFECT OF GARLIC

Nausea, vomiting and breath odor are major adverse effects especially when raw forms of the herb are used and care should be taken in consuming high quantities. Although garlic generally poses little in terms of safety issues, there are isolated cases of topical garlic burns and anaphylaxis.[67,77]

According to Tattelman, garlic should be taken with great caution in patients taking anticoagulants. It seems prudent to stop taking high dosages of garlic seven to ten days before surgery because garlic can prolong bleeding time.[78] One study indicated that garlic application usually results in local inflammation, but, if applied under a pressure bandage, or if there is poor wound care or a secondary infection, it also induces a severe dermal reaction. Data of a study showed that a high garlic dose induced liver toxicity and a pro-oxidative status characterized by increased malondialdehyde and decreased antioxidant enzyme activities as catalase, peroxidase, and superoxide dismutase. Another study suggested that garlic with a high dose has the potential ability to induce liver damage.[77,79]

A parallel study also highlighted the potential ability of a high dose of garlic to induce morphological changes in the liver and kidneys.[66] Another study also shows intraperitoneal (IP) administration of high doses of garlic (500 mg/kg) results in profound changes in lung and liver tissues of rats than oral administration. It is also shown that the adverse effect of high doses of garlic oil might further influence the hemostatic balance.[64,68]

CONCLUSION

A recent increase in the popularity of alternative medicine and natural products has renewed interest in garlic and their derivatives as potential natural remedies. Garlic, from crushed to capsules, and is consumed throughout the world. Garlic has a lot of benefits and potential uses in preventing and curing different diseases. Fresh and powdered garlic are popular for food flavor and should continue to be used. Nowadays, the problem of bacterial resistance is growing, and the outlook for the use of antibacterial drugs in the future is still uncertain. Even though pharmacological industries have produced a number of new antibiotics in the last few decades, resistance to these drugs by bacteria has increased. Garlic is a valuable source of new and biologically active molecules possessing antibacterial properties through direct action against bacteria or synergism with antibiotics. Garlic's antifungal, antibiotic and perhaps anticancer effects are well-accepted world over because of the many scientific literature supporting these effects. Garlic also has hepatoprotective, antioxidant, and anthelmintic effects as well as unidentified anti-malarial substances. In conclusion, a detailed study regarding the phytochemical assessment and pharmacological effect of garlic and more attention as well as researches regarding the anticoagulant, anti-inflammatory, immunomodulatory and wound-healing action. Besides, advances being made in analytical techniques, sophisticated bioassays, and biotechnological exploitation should provide the means by which these important plants continue to play a key role in the benefit of man and animals' health. Finally, medicinal plants are in danger due to marketing and using them for different activities, so every citizen should give care and conservation in their natural habitat, this can be achieved through public education to increase the awareness of the community about potential uses of medicinal plants.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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