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Exploring the Potential of Germinated Cowpea: A study on Nutrition, Functionality and Phytochemicals

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Abstract:

This research investigates the impact of germination on the chemical, functional, and anti-nutritional factors in cowpea (Vigna unguiculata L. Walp.) flour. Cowpea is a vital grain legume with significant economic and nutritional importance, particularly in less developed tropical regions. Germination, a simple and effective process, is explored for its potential to enhance the nutritional value of cowpea seeds. The study reveals that germination increases protein content and reduces fat and carbohydrate levels. Moreover, it significantly decreases anti-nutritional factors like tannins and phytic acid. The research also highlights the improved functional properties of germinated cowpea flour. The findings suggest that germination is a valuable technique for improving cowpea's nutritional quality and functionality, making it a promising ingredient for various food applications.

Keywords: Proximate composition, Functional properties, phytochemical screening,

1. Introduction:

Legumes, mainly the edible dry seeds, are one of the major classes of seeds that play an important role in human nutrition (Iqbal et al., 2006). Legumes are grown agriculturally, primarily for their grain seed called pulse, for livestock forage and silage, and as soilenhancing green manure. A legume fruit is a simple dry fruit that develops from a single carpel and usually dehisces (opens along a seam) on two sides (Messina, 1999).

Cowpea (*Vigna unguiculata L. Walp*.) with a chromosome count of 2n = 2x = 22 belongs to the *Phaseoleae* tribe within the Leguminosae family. This tribe comprises several economically significant warm-season grain and oilseed legumes, including soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*). The name "cowpea" likely originated from its historical use as a vital source of fodder for cattle in the southeastern United States and other regions worldwide. In various parts of West Africa, cowpea is known by names such as "niebe," "wake," and "ewa," while in Brazil, it's referred to as "caupi." In the United States, cowpeas go by various names like "southernpeas," "blackeyed peas," "field peas," "pinkeyes," and "crowders." These names reflect the traditional categorizations and market classes that evolved over time in the southern United States(Michael etal.,2007).

According to Food and Agriculture Organization (FAO) 2021, total world production of cowpeas in 2019 was 8.9 millon tons. According to Animasaun et al. 2015 worldwide production of cowpea is about 4.5 million metric tons. According to IITA 61% of production comes from Africa and 58% of production comes from worldwide. Not only seeds but also flowers and leaves are edible part of cowpea (Nwosu et al. 2021; Langyintuo et al. 2003)

The cowpea, is a grain legume native to Africa that has significant economic and social significance in developing nations. The cowpea is a food that is very important to many people, especially in less developed tropical nations, as it is the primary source of protein and carbohydrates for a big portion of the global population. Nutraceuticals such dietary fibre, antioxidants, polyunsaturated fatty acids (PUFA), and polyphenols are also abundant in cowpeas in addition to nutrients (Phillips et al., 2003; Trinidad et al., 2010; Shetty et al., 2013; Baptista et al., 2017).

Germination generally increases the nutritive value of seeds (Chen,1970). Germination is one of the simplest ways of improving the nutritional quality of seeds. Research by Chen et al. (1975) showed that nutrient/energy ratios for some vitamins, especially ascorbic acid, thiamin, riboflavin and niacin, in germinated seeds are higher than in the dried seeds. It is a good source of protein containing 18–35% (Aremu, 1990). The protein contents (N × 6.25) has been shown to be higher in germinated seeds than dry seeds (Chen et al.,1975).

The present study was aimed to investigate the nutritional composition, functional composition of germinated cowpea flour. This study also overviews the antinutrional factor and phytochemical screening of germinated cowpea flour.

2. Materials and Methodology:

A. Selection of ingredients:

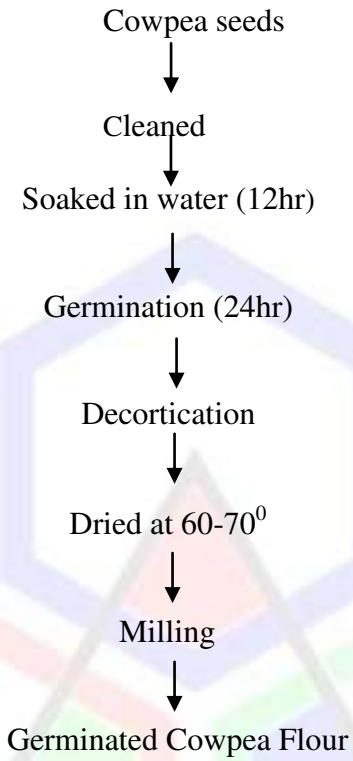
The legume crop Cowpea was selected as the major ingredient for the study. The selected raw material was procured from the local market of Parbhani. The raw material was visually inspected, and grains/seeds were cleaned from any extraneous matter.

B. Germination Process:

Initially, Germination process optimization by varying time conditions for steeping and germination of cowpea was the major focus of study. The cowpea were soaked initially in lukewarm water at the ratio of 1:5. The cowpea germination was performed by optimizing soaking time, and germination time and maintaininga germination temperature of 30 ^{o}C. The germinated

cowpea was further subjected to cabinet drying(55^{o}C). Further germinated cowpea sprouts were ground to flour and sieved to obtain fine flour powder.

Preparation of germinated cowpea flour:



C. Proximate analysis:

The nutritional parameters of germinated cowpea flour un comparison of raw cowpea flour was determined. The standard method determined the moisture content and ash content (AOAC, 1990). The determination of protein content was estimated by the Kjeldhal method by initially calculating nitrogen content and further protein from nitrogen content. The crude fat was extracted and measured by the Soxhlet apparatus using n-hexane, as per AACC, (2000). The crude fibers and total carbohydrates were performedaccording to the methodology described by Ranganna (2011).

D. Functional Properties:

Water and Fat absorption:

The Sosulski et al. (1976) method was used to estimate the water absorption capacity, and the Lin et al. (1974) method was used to determine the fat absorption capacity using a 4 g flour sample and refined palm oil (King's Brand, Devon Ind., Singapore; density 0.88 g/ml). The values were given as grams of oil or water absorbed by one gram of protein or grain.

Bulk Density:

Following Okaka and Potter's (1979) method, 50 g of cowpea flour were added to a 100 ml measuring cylinder and tapped to a consistent volume. The bulk density (gcm3) was then computed using the following formula:

Bulk density = weight of flour (g) / flour volume (cm 3).

E. Anti-nutritional factors:

The anti-nutritional factors were estimated for anti-nutrients such as phytates and tannins.

Raw Cowpea Seeds

Germinated Cowpea Seeds

Germinated Cowpea Flour

F. Qualitative analysis of Phyto-chemical:

The powder germinated cowpea samples (50 g/250 mL) were extracted successively with methanol, acetone and water using soxhlet apparatus at 55-85 ℃ for 8- 10 h in order to extract the polar and non-polar compounds (Elgorashi and Staden, 2004). For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 40C for further use .ethanol, aqueous extracts were used for preliminary phytochemical analyses using standard procedures (Harbone, 1973; Kokate et al., 1995). The following qualitative tests were used for germinated cowpea flourextract.

i. Test for Alkaloid

Wagner's test:

About ten mg of extract was taken and few drops of Wagner"s reagent (Dissolve 2 g of iodine and 6g of KI in 100 cm3 of water) were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

ii. Test for Flavonoids

Lead acetate test:

Ten mg of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

iii. Test for Phenolic compounds

Ferric chloride test

The extract was diluted to 5 ml with distilled water. To that few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the resences of phenolic compounds.

3. Result and Discussion:

A. Optimization for germination process

The data for process optimization of cowpea with respect to soaking time, germination time and percent germination yield (%) was calculated and presented in table 1. The simultaneous increase in germination time depicts the germination yield. Highest germination yield was observed in 24 hrs of germination

Table 1: Process optimization for germination process

Parameter	Soaking time (hr)	Germination Time(hr)	Percent Germination (%)
Optimization 1	12	0	0.00
Optimization 2	12	12	47.4
Optimization 3	12	18	71.1
Optimization 4	12	24	94.8

B. Proximate analysis of raw and germinated cowpea flour

Table 2 depicts differences in key nutritional components between raw cowpea and germinated cowpea flour. The moisture content in raw cowpea was 8.37% ± 0.07%, while germinated cowpea flour had a higher moisture content of 9.60% ± 0.04%, attributed to water absorption during soaking. Protein content in raw cowpea flour was 23.06% ± 0.05%, which increased to 24.5% ± 0.4% in germinated cowpea flour. In contrast, there was a significant reduction in fat content (from 2.1% ± 0.25% to 1.7% ± 0.75%) and carbohydrate content (from 60.93% ± 0.08% to 58.02% ± 0.09%) in germinated cowpea flour.

The ash content increased from 2.28% ± 0.65% in raw cowpea to 4.36% ± 0.37% in germinated flour. Notably, the fiber content decreased from 3.2% ± 0.3% in raw cowpea to 1.86% ± 0.45% in germinated flour, likely due to the enzymatic degradation of fiber into simpler sugars during germination, a phenomenon consistent with previous research (Desalegn B.B, 2015; Giami, 1992).

Table 2: Nutritional composition of Raw and Germinated cowpea flour

Parameter (%)	Raw cowpea flour	Germinated cowpea flour
Moisture	8.37±0.07	9.60 ±0.04
Protein	23.06±0.05	24.5±0.4
Fat	2.1±0.25	1.7±0.75
Carbohydrate	60.93±0.08	58.02±0.09
Ash	2.28±0.65	4.36±0.37

Mineral composition of Raw and Germinated cowpea flour:

The mineral content of germinated cowpea flour was found to be lowered after germination. The research study investigated the minerals in raw and germinated cowpea flour as calcium, magnesium, iron and zinc 54.46±0.35 to 55.3±0.3mg/100g, 88.23±0.25 to 85.66±0.25mg/100g, 17.3±0.2 to 19.3±0.28mg/100g, 4.7±0.2 to 4.66±0.20mg/100grespectively. A similar reduction in mineral content was found by Akinlosotu & Akinyele (1991). It may happen due to the leaching of minerals during soaking into the solvent i.e. water.

The increase in Phosphorus content in germinated flour from 461.33±3.51 to 480.66±2.08mg/100g. Increase in Phosphorus content during germination because of increased phytase activity during germination. Phytase breaks down phytic acid to phosphorous and significant increases in phosphorous were observed Singh & Banerjee (1953).

Table 3: Mineral composition of Raw and Germinated cowpea flour

Parameter (mg/100g)	Raw Cowpea Flour	Germinated cowpea flour
Calcium	54.46±0.35	50.3±0.3
Magnesium	88.23±0.25	85.66±0.25
Iron	17.3±0.2	19.3±0.28
Zinc	4.7±0.2	4.66±0.20
Potassium	1235.67±4.16	1190±4
Phosphorous	461.33±3.51	480.66±2.08

C. Functional Properties of Raw and Germinated Cowpea Flour:

Table 4 depicted that the Bulk density was decreased in the germinated cowpea flour than raw cowpea flour 0.59± 0.02, 0.62 ± 0.03. Water absorption capacity, Oil absorption capacity was observed to increase in germinated cowpea flour than raw cowpea flour i.e.1396.33 ± 6.02, 1264.33 ± 5.13, and 1185.66 ± 3.05, 921 ± 2 respectively.The rise in water-absorbing capacity during germination can be linked to three factors: an increase in protein quantity, improvements in protein quality as germination progresses, and the breakdown of polysaccharide

molecules. The reason germinated samples can absorb more fat is because they have more of these amino acids available on the surface. Ghavidel & Prakash (2006)

Table 4: Functional properties of Raw and Germinated cowpea flour

Parameters	Raw cowpea flour	Germinated cowpea flour
Tannin (mg/100g)	215.07±0.04	187.23±0.04
Phytic acid (g/100g)	4.75±0.07	3.24±0.05

D. Anti-Nutritional Factor:

From the table 5, it concluded that the tannin content in raw cowpea flour and germinated cowpea flour is 215.07±0.04mg/100g and 187.23±0.04 mg/100g respectively. Similarly, the phytic acid content in raw and germinated cowpea flour was determined to be 4.75±0.07g/100g and 3.24±0.05g/100g, Germination process decreases the anti-nutritional factor present in cowpea. Results are close finding with Ibrahim et al., (2002). The reduction during germination was due to consumption of phytates in growth. The tannin are water soluble compound mainly present in seed coat of cowpea, hence it get lost in water during steeping and dehulling after germination and drying.

Table 5: Antinutritional factor in Raw cowpea and Germinated Cowpea Flour

Parameter	Raw Cowpea flour	Germinated Cowpea flour
Bulk Density	0.62 ± 0.03	0.59 ± 0.02
Water absorption capacity	1264.33 ± 5.13	1396.33 ± 6.02
Oil absorption capacity	921 ± 2	1185.66 ± 3.05

E. Qualitative test for germinated cowpea flour:

Phytochemical constituei	Presence (+) or Absence (-) in different extract	
	Aqueous	Ethanolic
Phenol	+	+
Alkaloid	+	-
Flavanoid	+	+

Table 6. Qualitative phytochemical analysis of Germinated cowpea flour

A qualitative assessment was conducted on germinated cowpea flour to determine the presence of bioactive compounds, also known as phytochemical constituents or secondary

metabolites. Initial phytochemical investigations underscore the significance of isolating natural compounds from their mixtures, as these isolates can find applications in a range of clinical practices Ganatra et al., (2013). The qualitative analysis results for Germinated cowpea flour phytochemical components are summarized in Table 6.

Through phytochemical tests, it was noted that different extracts, including ethanol and, water extracts, displayed varying levels of phytochemical constituents. Specifically, the ethanol extract exhibited the presence of phenols and, flavonoids. On the other hand, the aqueous extract yielded positive results for alkaloids, phenols, and flavonoid. The detection of these phytochemical constituents in the germinated flour extracts suggests the potential presence of nutraceutical properties.

The study revealed that the germinated cowpea flour is a valuable source of diverse secondary metabolites, each known for its protective and therapeutic properties. For example, alkaloids offer protection against chronic illnesses.

Similar findings were detected by Jaya et al., (2019) who reported the phytochemical analysis of Vigna Unguiculata seeds such as Alkaloids, Flavonoids, Terpenoids, Tannins, Steroids, phenols, Glycosides and Saponin in different solvent extracts such as acetone, chloroform, Ethanol and Aqueous.

4. Conclusion:

The study focused on the impact of germination on the chemical, functional, and anti-nutritional factors in cowpea. It revealed that germination increased the protein content and decreased fat and carbohydrate levels in cowpea flour. Additionally, germination reduced fiber content, likely due to enzymatic breakdown. The mineral content, including calcium, magnesium, iron, and zinc, decreased in germinated cowpea flour, possibly due to leaching during soaking. However, phosphorus content increased, attributed to enhanced phytase activity. Functional properties like water and fat absorption capacities improved in germinated cowpea flour, making it more suitable for various food applications. Furthermore, the study showed a reduction in anti-nutritional factors, such as tannins and phytic acid, during germination. These findings indicate that

germinated cowpea flour has enhanced nutritional quality and reduced anti-nutritional factors, making it a potentially valuable ingredient in food production.

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