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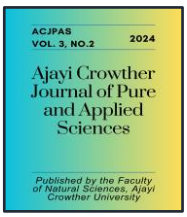

Article

Comparative studies on the influence of extraction methods on amino acid and functional properties of black benniseed (*Sesamum radiatum*) and false sesame (*Ceratotheca sesamoides*) protein concentrates

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Abstract

Usage of plant proteins for development and formulation of functional food products has taken centre stage. In this study, functional parameters and amino acid profile of protein concentrates extracted from false sesame and black benniseed were appraised. The extraction was carried using isoelectric precipitation and ammonium sulphate precipitation methods. The protein yield was higher for the Isoelectric precipitation method (28.25-33.99%) than ammonium sulphate (13.36-30.72%). In the same vein, isoelectric precipitated concentrates had higher protein content (76.17 and 68.60%) than ammonium sulphate concentrate (60.02 and 65.8%). However, for functional properties ammonium sulphate concentrate gave improved functional parameter results in terms of water absorption capacity (208.24-225.62%), oil absorption capacity (175.60-218.95%), and swelling capacity (184.83-219.79%). The total essential, non-essential, hydrophobic, and hydrophilic amino acids content was higher in concentrates gotten from ammonium sulphate precipitation method than the concentrates gotten from isoelectric precipitation method. Hence, our findings suggest that ammonium sulphate method may be the most applicable method for promoting enhanced functionality and utilization of false sesame and black benniseed protein concentrates.

Keywords: False sesame seed; black benniseed; isoelectric precipitation; ammonium sulphate precipitation; amino acids.

1. Introduction

Black Benniseed (*Sesamum radiatum*) and false sesame (*Ceratotheca sesamoides*) are distributed globally and consumed as a leafy vegetable [1]. The genus *Sesamum and Ceratotheca* is of the Pedaliaceae family. Generally, the family comprises of 16 genus, 60 annual and perennial species among which black Benniseed is one of the most important [2]. Black Benniseed is widely used in Nigeria and has also played an important role in Nigerian export [3]. Black Benniseed contains 50% of oil and 25% of protein with a combined unique balance of essential amino acid rich in methionine, tryptophan and cysteine. The absence of these type of amino acids from common sources of vegetable protein such as soya bean protein makes the legume seed a more unique one [4].

False sesame (oil seed crop) is grown for its edible oil, vitamins, protein and amino acids [5]. They are good source of protein which ranged from 15-33% [6,7] and are poorly researched and fall within the

underutilized species in Africa. Furthermore, they play significantly role in Republic of Benin (center and North) as a daily need and nutritional requirements of the people [1,6,8,9]. In addition, False sesame has been reported in the management of cardiovascular diseases and aids bowel digestion and childbirth [10].

Presently, research has shifted to usage of vegetable proteins for new product development and formulation of functional foods. This is because animal proteins are expensive especially in developing countries when compared to plant proteins [11]. Plant proteins concentrate maybe useful as improvement for nutritional as well as functional quality in food products. The usage of plant protein concentrates in food formulations has become of interest due to greater sustainability and lower production costs [12]. Depending on grain diversities and means of extraction, protein's yield, the composition and its functionality sometimes vary [13]. For instance, Arise *et al.* [13] used salt solubilization and acid precipitation method to investigate the functional parameters of South Africa Bambara groundnut landrace protein concentrate. The results show that the functional properties were greatly influenced by the extraction methods rather than the landrace. In the salt solubilization protein prepared, the values obtained for water and oil absorption capacity were two times greater than the values obtained for samples prepared through acid precipitation. In addition, Adebowale *et al*. [14], reported increment in the foaming capacity of Bambara protein isolate obtained through micellization methods over the ones obtained through isoelectric precipitation. Besides the extraction methods influencing the functional properties, it has also been reported that extraction methods also influenced the yield of protein in soya bean protein concentrates [15]. For instance, the yield of protein in soya bean concentrate (16.2%) obtained by micellization extraction was reported to be three to four times significantly lower than that gotten by isoelectric precipitation [15-17]. Thus, suggesting that extraction methods may impact the functionality of proteins, the protein yield and as a result, influence food applications.

There is little or no information on the influence of extraction methods on physicochemical and functional parameters of False sesame as well as Black Benniseed protein concentrates. Therefore, this study aims at investigating the functional parameters of protein concentrate extracted from false sesame and black benniseed seed flour using isoelectric precipitation and ammonium sulphate precipitation method.

2. Methods

2.1 Materials

The seeds of false sesame and black benniseed were purchased from Oja-Oba market in Ilorin, Kwara State, Nigeria. They were identified and authenticated at the Herbarium unit, Botany Department, University of Ilorin, Kwara State, Nigeria and given voucher number, UILH/002/1367 and UILH/003/1367 respectively.

2.2 Preparation of defatted flours

The seeds were dehulled, grounded using a blender and sieved to fine powder. Using n-hexane the seed flours were defatted at 60^{o}C (boiling point of the solvent is roughly 69^{o}C and components of interest are neither volatile nor biodegradable at that temperature) for 8 hours using Soxhlet apparatus. The defatted flours were air-dried at room temperature and stored in air-tight containers prior to laboratory analysis.

2.3 Preparation of the concentrates

With slight modification, the method adopted by Adebowale *et al.* [14] was used for the preparation of the isoelectric precipitation concentrates. Briefly, the defatted flour in the ratio 1:20 (flour: water ratio) was suspended in water at a pH of 6.37. The slurry obtained was agitated for 2 h (using 1 M NaOH for the desired pH of 9.0 adjustment), centrifuged using automated super speed refrigerated centrifuge for 30 min at 10,000 × g at 5^{0}C. Following the centrifugation and recovery process, the resulting supernatant was pooled and the proteins precipitated at isoelectric point (IP) pH of 5.0. Subsequently the protein

precipitate formed was recovered by centrifugation at 10,000 × *g*, 5⁰C after 15 min. The obtained protein concentrate was freeze-dried.

For ammonium sulphate precipitation, the method of Karaca *et al*. [18] was used with minor modifications. Briefly, defatted flour (100 g) was mixed with 5% ammonium sulphate aqueous solution at 1:10 ratio (w/v), adjusted to pH 7.00 with 0.1 M NaOH and stirred at 500 rpm for 1 hr at room temperature. The slurry was centrifuged at 17,700 ×g for 20 min at 4°C. The resulting supernatant was collected, dialyzed using Milli-Q™ water severally at 4 °C for 72 hrs until conductivity reached 2.0–2.5 mS/cm. The resulting supernatant was freeze-dried at 30 °C and stored prior to analysis.

2.4 Protein yield and content

The method of Arise *et al.* [19], was adopted for the calculation of protein yield, determined as protein concentrate (dry weight) after precipitation and solubilization divided per weight of the defatted flour as shown below. The protein content (N × 6.25) of the protein concentrates were determined by Kjeldahl method [19].

$$\text{Protein Yield (\%)} = \frac{\text{Protein concentrate recovery} \times \text{Protein content of concentrate (\%)}}{\text{Protein content of defatted flour (\%)}} \times 100 \qquad (1)$$

2.5 Amino acid composition

Pico-Tag method [20] was adopted for the Amino acid content determination. Briefly, 2.0 g of the sample was hydrolysed, concentrated using a rotary evaporator and loaded into Technicon Sequential Multi-Sample Amino Acid Analyser (TSM-1) (Technicon Instruments Corporation, New York, USA). Each hydrolysate (10 µL) was dispensed into the cartridge of the analyser, separated and analysed for free acidic, neutral and basic amines, which lasted for 76 hrs using Norleucine as the internal standard. A 10 µL standard solution mixture of amino acid was also loaded into the analyser. The results of the standard and samples was printed out as chromatogram peaks.

Calculation from the peaks: Each chromatogram's peak height, half-height and the width of the peak at half-height was measured. Approximate area of each peak was then obtained by multiplying the height with the width of the half height. All measurements were in millimetre (mm). The neulocine equivalent (NE) for each amino acid in the standard mixture was calculated as:

$$NE = \frac{Area\ of\ neulocine\ peak}{Area\ of\ each\ amino\ acid\ in\ the\ standard\ mixture} \qquad (2)$$

The predicted protein efficiency ratio (P-PER) values from their amino acid composition were calculated based on the equation adopted by [21] and [22] as given below:

$$P\text{-}PER = -0.468 + 0.454(Leu) - 0.105(Tyr) \qquad (3)$$

2.6 Evaluation of Functional properties

2.6.1 Solubility index

With slight modification the concentrates solubility index was determined using the method adopted by [14]. 0.2 g of the concentrates was dissolved in 100 g distilled water, using either 1 M HCl or 1 M NaOH the pH was adjusted accordingly to 11. The resulting sample suspensions were shaken for 30mins (room temperature) and centrifuged at 4000 /g for a period of 30mins. Micro Kjedahl method [19] was used in determining the percentage nitrogen in each supernatant [19]. Percent nitrogen multiplied by 6.25 was used in calculating percent soluble protein on wet basis.

2.6.2 Least gelation concentration

The method described by Almanza-Benitez *et al*. [23] was used for the determination least gelation concentration. Sample suspensions of each protein concentrates 2%-20% (w/w) were prepared using distilled water and the resulting dispersions poured into test tubes. After which the test tubes were

heated for 1hr in boiling water and rapidly cooling using a water bath and further cooling to 4°C for 2 hrs was carried out. The sample that did not slide down the test tubes when upturned had the least gelation.

2.6.3 Water and oil absorption capacity

Water absorption capacity was determined using the modified method of [14]. To 0.5 g of the sample in a beaker, 10 ml of distilled water was added and agitated for 5 mins. The obtained suspension was centrifuged for 30 mins at 4000 × g. Subsequently, the resulting supernatant was measured using a 10-ml graduated cylinder. Water density was taken as 1.0 g/cm^3. The water absorbed was then calculated as the difference between the initial volume of water added to the sample and the supernatant volume. For oil absorption capacity determination similar procedure was employed except that instead of water, groundnut oil was used.

2.6.4 Swelling capacity

The swelling capacity was determined by adopting the method described by Ayodele and Beatrice [24]. The protein concentrates were filled into a 100 ml graduated cylinder to the 10 ml mark and distilled water added to make it up to a total of 50 ml. The graduated cylinder was tightly covered and mixed by inversion. After 2 min, the cylinder was inverted again. After 8 mins, the volume occupied by the sample was taken and expressed as the swelling capacity

$$SI = \frac{volume\ after\ soaking - volume\ before\ soaking}{Weight\ of\ sample} \qquad (4)$$

Where SI is swelling index.

3. Results

3.1 Protein yield and content of the protein concentrates

The isoelectric precipitation (IEP) method used in the extraction of the concentrates gave higher protein yields (76.17% and 68.6%) compared to the protein content (60.20% and 65.8%) obtained by ammonium sulphate (Fig. 1). The protein content of False sesame and Benniseed protein concentrates obtained in this study was lower than that previous study for pigeon pea isoelectric-precipitated isolates (82.4%), pigeon pea micellization-precipitated isolates (82.8%), and hemp seed isoelectric-precipitated isolates (84.15%). However, it was higher than Bambara groundnut salt solubilization concentrates (57%). Differences in extraction materials and protein precipitation methods may account for the variations in protein content.

The method of extraction influenced the amino acid composition. Protein concentrates prepared using the ammonium sulphate method gave higher composition results compared to the concentrates prepared by isoelectric precipitation. The total essential amino acids were greater in sample D (43.88 g/100 g protein) than in sample A, B, and C (36.89, 40.27, 33.22 g/100 g protein, respectively) (Table 1). The total percentage of essential amino acids and non-essential amino acids of the concentrates obtained from isoelectric precipitation were higher than those obtained through ammonium sulphate precipitation because of the change in pH during isoelectric precipitation. The amino acids containing sulphur (methionine and cysteine) were higher in ammonium sulphate concentrates due to the higher value of methionine and cysteine. The ammonium sulphate concentrates had higher values of total percentage of hydrophobic amino acids, resulting in lower water absorption capacity, while there was a higher value of total percentage of hydrophilic amino acids for isoelectric precipitation, implying higher water absorption capacity. The most abundant amino acids found in false sesame and black benniseed protein concentrates were the acidic amino acids (aspartic and glutamic acid).

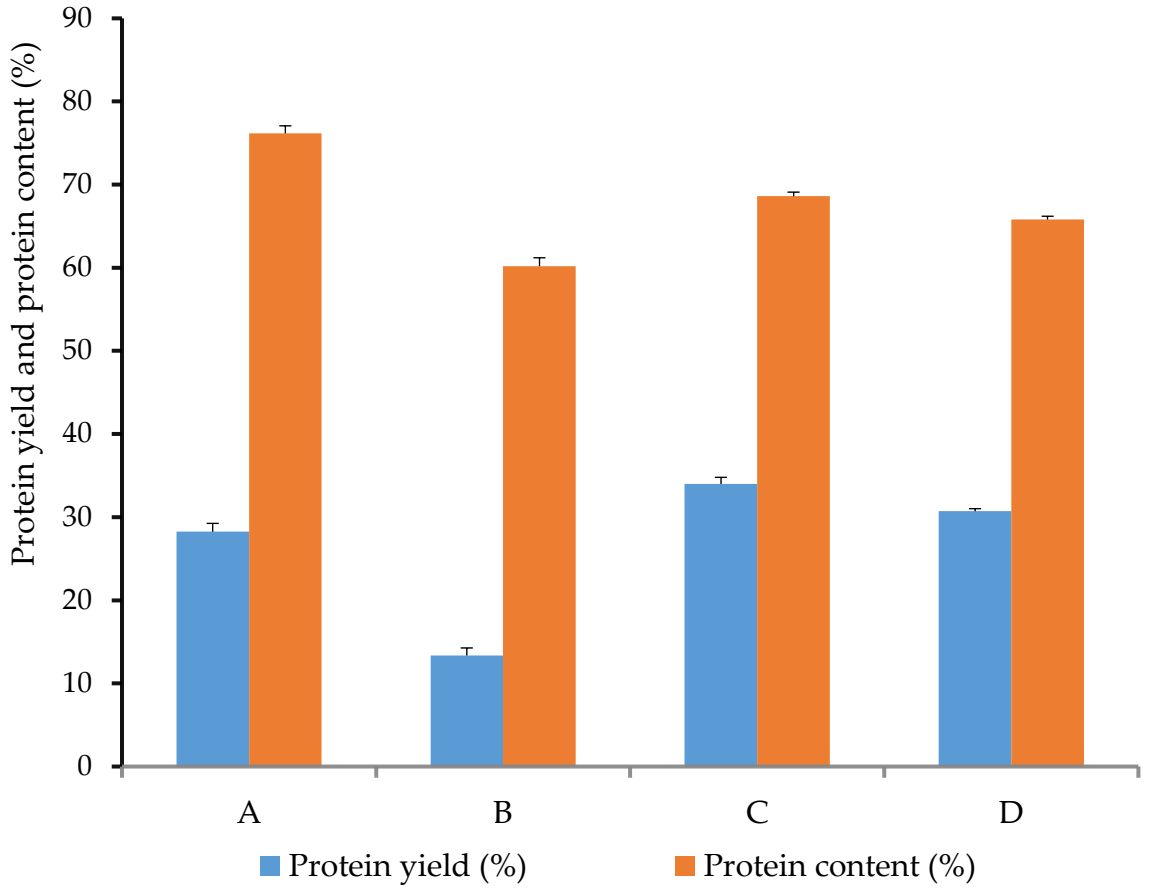

Figure 1: Protein content and protein yield of the protein concentrates**.** *A: black benniseed, Isoelectric concentrate, B: black benniseed, Ammonium Sulphate concentrate, C: false sesame, Isoelectric concentrate, D: false sesame, Ammonium sulphate concentrate.*

3.2 Functional properties of false sesame and black benniseed protein concentrate

3.2.1 Solubility Index

The results of the study indicate that the solubility profile of proteins during the isolation process provides valuable insights into irreversible aggregation, denaturation, and precipitation that may have occurred (Table 2). This parameter is particularly important in the food industry as it can significantly influence properties such as gelation, emulsification, and foaming.

The extraction methods employed in the preparation of protein concentrates had a significant impact on their solubility index. Comparing the protein concentrates obtained through the isoelectric precipitation method and those prepared using ammonium sulfate, it was observed that the concentrates obtained through isoelectric precipitation exhibited higher solubility values across all temperatures. The higher solubility values observed in the protein concentrates prepared through isoelectric precipitation can be attributed to the presence of hydrophobic residues in minute quantities and a high charge level. This suggests that these factors contribute to the enhanced solubility of the concentrates.

This finding is further supported by the lower hydrophobic values observed in the concentrates prepared through the isoelectric precipitation method, as indicated in Table 2. The concentration of hydrophobic residues in the protein concentrates can directly influence their solubility, and a lower hydrophobic value suggests a higher solubility. However, despite the improved solubility observed in the protein concentrates prepared through the isoelectric precipitation method, there are still limitations to their application as functional constituents.

Table 1: Amino acid composition of false sesame and black benniseed protein concentrates

Amino acids	Sample A(g/100g)	Sample B (g/100g)	Sample C (g/100g)	Sample D (g/100g)	[1]FAO (children)[1*]	[1]FAO (Adult)[1*]
Essential amino acid						
Histidine	2.25	2.44	1.90	3.02	1.90	1.60
Valine	4.00	4.75	3.31	5.01	3.50	1.30
Methionine	1.92	2.23	1.56	2.55	2.50	1.70
Tryptophan	1.26	2.00	1.04	2.10		
Isoleucine	4.91	5.00	4.16	5.24	2.80	1.30
Leucine	7.67	7.81	6.91	8.21	6.60	1.90
Phenylalanine	5.94	6.03	4.80	6.20	6.30	1.90
Threonine	4.02	4.75	4.50	5.35	3.40	0.90
Lysine	4.93	5.26	5.04	6.20	5.80	5.00
Total essential amino acid	36.90	40.27	33.22	43.88		
Non – essential amino acid						
Tyrosine	3.10	4.50	3.90	5.22		
Cysteine	0.36	1.23	0.56	1.83		
Aspartic acid	9.92	10.66	8.74	12.31		
Glutamic acid	13.62	14.30	12.20	16.77		
Serine	4.80	5.01	4.02	4.96		
Glycine	2.36	3.41	3.06	4.32		
Arginine	6.81	7.40	7.06	8.20		
Alanine	3.01	3.84	4.01	4.72		
Proline	4.21	4.54	3.95	4.82		
Total non-essential amino acid	48.19	54.89	47.50	63.15		
Amino acids with different charcterization[2*]						
Basic	13.99	15.10	14.00	17.42		
Acidic	23.54	24.96	20.94	29.08		
Hydrophobic	31.66	34.20	28.70	36.75		
Hydrophilic	46.34	49.82	43.46	56.82		
P-PER	2.69	2.61	2.26	2.71		

A: black benniseed, Isoelectric concentrate, B: black benniseed, Ammonium Sulphate concentrate, C: false sesame, Isoelectric concentrate, D: false sesame,

Ammonium sulphate concentrate [1]FAO (2007)

P-PER- Predicted protein efficiency ratio

[2]Basic: lysine, arginine, histidine; acidic: aspartic acid, glutamic acid; hydrophobic: alanine, isoleucine. Methionine, phenylalanine, valine, proline; Hydrophilic Arginine, glutamic acids, histidine, lysine, serine threonine

Table 2: The solubility index of false sesame and black benniseed protein concentrates at different temperatures

Samples	Solubility index at 40°C	Solubility index at 60°C	Solubility index at 80°C
A	91.12	50.31	61.12
B	21.80	23.90	25.32
C	71.91	70.22	74.80
D	57.12	39.61	8.00

A: black benniseed, isoelectric concentrate, B: black benniseed, ammonium Sulphate concentrate, C: false sesame, isoelectric concentrate, D: false sesame, ammonium sulphate concentrate

3.2.2 Least Gelation Concentration (LGC) and Swelling capacity

An important functional property of food proteins is their ability to form gels when heated during food formulation and processing. The minimum protein concentration at which the gel does not slip down the test tube walls in an upturned position is known as the LGC. Lower LGC values indicate better gelling ability.

In this study, the concentrates with the least gelation were not affected by the extraction method or plant varieties, as shown in Table 3. This indicates that these concentrates exhibited consistent gelation properties regardless of the extraction method or the specific plant varieties used.

Furthermore, the study evaluated the swelling capacity (SW) of the protein concentrates. Swelling capacity refers to the increase in volume that occurs when a sample absorbs water. It is a well-known parameter in food processing, as changes in volume can impact the acceptability of the final product. Table 4 presents the results of the swelling capacity analysis. Interestingly, the extraction method had a significant influence on the swelling capacity of the concentrates, while the plant varieties had minimal impact. Specifically, the concentrates prepared through the ammonium sulfate method exhibited higher swelling capacity compared to those obtained through the isoelectric precipitation method.

3.2.3 Water absorption capacity (WAC)

Table 4 shows the results on influence of extraction methods on WAC. The WAC of the ammonium sulphate ranged from 203.9% to 225.9% while the isoelectric protein concentrates protein concentrates ranged from 21.3% to 80.9%.

3.2.4 Oil absorption capacity (OAC)

Oil absorption capacity, involves the binding of fat by non-polar side chain of proteins and is an hint of whether the food sample or protein material will do well as a meat extender or analogues (Ayodele and Beatrice, 2015). In this study, the methods of extraction influenced OAC rather than the varieties (Table 4).

Table 3: The least Gelation Concentration of false sesame and black benniseed protein concentrates at different temperatures

Samples	40^{0}C	60^{0}C	80^{0}C
A	-	-	+
B	-	-	+
C	-	-	+
D	-	-	+

A: black benniseed, Isoelectric concentrates, B: black benniseed, Ammonium Sulphate concentrates, C: false sesame, Isoelectric concentrates, D: false sesame, Ammonium sulphate concentrates

Table 4: Water Absorption, Oil absorption and Swelling capacity of the protein concentrates

Samples	WAC (%)	OAC (%)	SW (%)
A	22.57^{d}±0.20	71.50^{d}±0.30	21.04^{d}±0.05
B	208.24^{b} ±0.50	175.60^{c} ±0.25	184.83^{b} ±0.20
C	80.09^{c}±0.55	193.24^{b}±0.02	104.15^{c}±0.11
D	225.62^{a} ±0.50	218.95^{a} ±0.40	219.79^{a} ±0.54

Mean values ± SD of double analysis, $P < 0.05$
A: black benniseed, Isoelectric concentrate, B: black benniseed, Ammonium Sulphate concentrate, C: false sesame, Isoelectric concentrate, D: false sesame, Ammonium sulphate concentrate
WAC-water absorption capacity, OAC-oil absorption capacity, SW-swelling capacity

4. Discussion

For the improvement of nutritional and functional attributes of many food products plant protein concentrates has been considered. Variations in protein yield and content were observed due to the different plant varieties and extraction methods used. The higher protein yields obtained through the isoelectric precipitation (IEP) method compared to the ammonium sulphate method can be attributed

to the pH changes during the IEP method, which enhance protein extractability. This finding is consistent with a study by Karaca *et al*. [18], where isoelectric precipitation resulted in higher protein yields for chickpea, faba bean, and lentil compared to salt extraction methods. Similarly, Malomo *et al*. [25] reported higher protein content and yield for hemp seed protein isolates prepared using the isoelectric precipitation method. Furthermore, Arise *et al*. [11] found that Bambara protein concentrates prepared by acid precipitation exhibited higher protein yield and content compared to concentrates prepared by salt solubilization. These results suggest that the choice of extraction method can significantly affect protein yield and content. It is worth noting that the protein content of False sesame and Benniseed protein concentrates in this study was lower than previously reported values for other protein sources. This discrepancy could be attributed to variations in extraction materials and protein precipitation methods employed in different studies.

The results revealed that the method of extraction significantly influenced the amino acid composition of the protein concentrates. The ammonium sulphate method yielded higher composition results compared to isoelectric precipitation. This finding is consistent with previous studies by Adebowale *et al*. [14] and Arise *et al*. [26]. The higher values of total essential amino acids obtained from isoelectric precipitation indicate its effectiveness in preserving these essential components.

The differences in amino acid composition between the two extraction methods can be attributed to the pH changes during the isoelectric precipitation process. The concentrates obtained from isoelectric precipitation showed higher percentages of essential and non-essential amino acids. Furthermore, the presence of sulphur-containing amino acids (methionine and cysteine) was more prominent in the ammonium sulphate concentrates. This variation in amino acid content suggests that the choice of extraction method can impact the nutritional profile of the protein concentrates.

The observed differences in hydrophobic and hydrophilic amino acid percentages between the extraction methods have implications for the water absorption capacity of the concentrates. Ammonium sulphate concentrates, with higher values of hydrophobic amino acids, exhibited lower water absorption capacity, whereas isoelectric precipitation concentrates, with higher values of hydrophilic amino acids, displayed greater water absorption capacity. The predominant presence of acidic amino acids (aspartic and glutamic acid) in the false sesame and black Benniseed protein concentrates aligns with previous findings for Bambara protein isolates [14] [26] and African yam bean protein [22]. The higher levels of glutamic acid and aspartic acid in the ammonium sulphate concentrates compared to the isoelectric precipitation concentrates can be attributed to the pH changes during the isoelectric precipitation process.

Additionally, the predicted protein efficiency ratio (P-PER) values, which indicate the growth-promoting value of protein, varied between the extraction methods. The ammonium sulphate concentrate of false sesame seed had a higher P-PER value (2.71) than the isoelectric precipitated false sesame seed protein concentrate (2.26). This suggests that the extraction method influences P-PER more significantly than the varieties. The obtained P-PER values indicate that both extraction methods produced protein concentrates of good quality, as they exceeded the values endorsed by FAO/WHO for both children and adults [27].

Protein solubility plays a crucial role in determining the functionality and quality of protein concentrates. The solubility profile obtained during the isolation process provides valuable insights into the extent of irreversible aggregation, denaturation, and precipitation that may have occurred. Such information is particularly relevant in the food industry, as protein solubility can significantly impact various properties, including gelation, emulsification, and foaming.

The extraction methods used in the preparation of protein concentrates have a direct influence on their solubility index. In this study, the researchers observed that protein concentrates obtained through the isoelectric precipitation method exhibited higher solubility values compared to those prepared using ammonium sulphate. These findings suggest that the presence of hydrophobic residues in minute quantities and a high charge level contribute to the enhanced solubility of the concentrates obtained through the isoelectric precipitation method. Interestingly, the concentrates prepared via this method also exhibited lower hydrophobic values.

However, despite the improved solubility observed in the protein concentrates prepared through the isoelectric precipitation method, there are still limitations to their application as functional constituents. One significant hindrance is the limited solubility of these concentrates, which has been highlighted in previous studies [28]. This limitation restricts their potential use in various food applications, where solubility is a critical parameter for achieving desirable functional properties.

The solubility profile of protein concentrates obtained through different extraction methods plays a crucial role in determining their functionality. The results indicate that the isoelectric precipitation method yields concentrate with higher solubility values, likely attributed to the presence of hydrophobic residues in minute quantities and a high charge level. However, despite these improvements, the limited solubility of protein concentrates remains a challenge for their broader application as functional constituents in the food industry. Further research and development efforts are needed to overcome this limitation and fully exploit the potential of protein concentrates.

Gelation is an important functional property of food proteins, and the minimum protein concentration required for gel formation, known as the LGC, is a crucial parameter. Lower LGC values indicate better gelling ability [29]. In this study, the concentrates with the least gelation were not affected by the extraction method or plant varieties. This suggests that the gelation properties of these concentrates were consistent regardless of the extraction method or the specific plant varieties used.

In addition to gelation, another important functional property of protein concentrates is their swelling capacity (SW). Swelling capacity refers to the increase in volume that occurs when a sample absorbs water. This parameter is particularly relevant in food processing, as changes in volume can impact the acceptability of the final product [30]. Interestingly, in this study, the swelling capacity was influenced by the extraction method rather than the plant varieties.

The results of this study are consistent with a previous report by Arise *et al*. [11], which also found that the extraction methods had a greater influence on the functional properties of protein concentrates than the specific plant varieties used. Specifically, the concentrates prepared through the ammonium sulfate method exhibited higher swelling capacity compared to those obtained through the isoelectric precipitation method.

The results suggest that the extraction methods employed have a significant impact on the functional properties of protein concentrates. The concentrates with the least gelation were not influenced by the extraction method or plant varieties. Furthermore, the swelling capacity of the concentrates was influenced by the extraction method, with the concentrates prepared through the ammonium sulfate method exhibiting higher swelling capacity. These findings highlight the importance of selecting appropriate extraction methods to obtain protein concentrates with desired functional properties for specific food applications.

Protein concentrates prepared through isoelectric precipitation absorbed lesser water than those prepared through ammonium sulphate. This can be related to the result obtained in amino acids profile, that ammonium sulphate concentrate had higher value hydrophilic amino acids which influence the WAC. This same trend of lower WAC for IEP had been found in literature for desi and kabuli chickpea, yellow pea as well as red and green lentil concentrates [31]. In the same vein, lower water absorption capacity was reported for Bambara protein concentrate prepared through acid precipitation [11].

Therefore, the false sesame and black benniseed protein concentrates prepared through ammonium sulphate extraction method could be useful in promoting the water binding capacity of some food products like sausages and dough.

The OAC of the concentrates prepared through ammonium sulphate is higher than concentrates prepared through isoelectric precipitation method. This result is in line with the OAC reported for Bambara groundnut landrace concentrates which shows a lower OAC for acid precipitation method [11]. Additionally, a low OAC for isoelectric protein isolate was reported by Adebowale *et al.* [14].

These findings indicate that the extraction methods play a significant role in determining the oil absorption capacity of the protein concentrates, rather than the specific varieties used. The higher oil absorption capacity observed in the concentrates prepared through ammonium sulphate extraction suggests their potential usage for flavor retention, improvement of palatability, and extension of shelf life.

5. Conclusions

False sesame and black benniseed legume concentrates are good sources of protein. Methods of extraction influenced the functional properties as well as the amino acids composition of the black benniseed and false sesame rather than the varieties. Isoelectric precipitated concentrates of black benniseed and false sesame produce high protein yield and content compared to the concentrates prepared using ammonium sulphate. Using ammonium sulphate precipitation method, the protein concentrates prepared revealed better functional properties; water absorption capacity and oil absorption capacity as well as swelling capacity and solubility. This study proposed that ammonium sulphate extraction may be the most applicable method for promoting functionality and utilization of false sesame and black benniseed protein concentrates to most cereals and viable raw material as a complementary protein source for the food industries.

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