

Functional properties of edible mushroom *Astraeus hygrometricus*

M. Pavithra, K.R. Sridhar* and A.A. Greeshma

Department of Biosciences, Mangalore University, Mangalagangothri, Mangalore 574 199, Karnataka, India

*Corresponding author Email: kandikere@gmail.com

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ABSTRACT

This study provides functional attributes of uncooked and cooked edible tender fruit bodies of an ectomycorrhizal gasteroid mushroom *Astraeus hygrometricus* sampled from the Western Ghats of India. The pH-dependent protein solubility was highest at pH 10 in uncooked ($p < 0.01$) and cooked samples with least solubility at pH 8 and pH 4, respectively. The least gelation concentration was 18% and 20% in uncooked and cooked samples, respectively. The water-absorption capacity was higher in cooked samples ($p < 0.01$), whereas it was opposite for the oil-absorption capacity ($p > 0.05$). Emulsion activity was higher in uncooked samples, while emulsion stability was higher in cooked samples ($p > 0.05$). The cooked samples showed high foam capacity ($p < 0.01$) as well as foam stability ($p < 0.001$). The principal component analysis (PCA) depicted that the proximal components like total lipids and crude fibre contents have major role in controlling the functional properties. The functional properties of uncooked as well as cooked tender fruit bodies of *A. hygrometricus* have selective advantages and serve as valuable raw material in production of functional foods and pharmaceutical products with desired qualities.

Keywords: Non-conventional food, proximal features, functional foods, ectomycorrhizae, Western Ghats

INTRODUCTION

Protein-energy malnutrition (PEM) is one of the major concerns in developing countries owing to population explosion and expensive animal-derived foods (FAO, 2000). Similar to wild legumes, edible mushrooms although non-conventional serve as valuable food sources owing to their high protein, high fibre, high carbohydrate and low fat contents. Moreover, edible mushrooms are also endowed with bioactive components which possess the capacity to combat many human ailments. Several mushrooms are cultivated worldwide to meet nutritional and health requirements and many edible wild mushrooms are consumed based on traditional or indigenous knowledge of native people. Lignin-based solid wastes are the basic raw materials for cultivation of edible mushrooms. Tangible utilization of cultivated or wild mushrooms depends on their nutritional, bioactive and functional attributes. Utilization of mushroom proteins rely on functional properties, which are important in processing, formulation, storage, sensory evaluation and consumer acceptability. Functional properties of mushrooms depend on various factors like proximal qualities (e.g. proteins, carbohydrates, fats and fibre), bioactive principles (e.g. minerals, vitamins and beta-glucan) and conditions of processing (e.g. temperature, pH and ionic strength).

The Western Ghats of India is known for a variety of wild mushrooms, which have been recognized as source of human nutrition by traditional or indigenous knowledge of tribals and native people. Many wild mushrooms are available in large quantities and sold in local markets (Pahlevanlo and Janardhana, 2012; Karun and Sridhar, 2013). For instance, termite mound mushroom *Termitomyces mammiformis* has been collected up to 2000 kg in the forests of Alanahalli and Kushalnagar of the Western Ghats costing between US\$ 1.5-2/kg (mature) and US\$ 4-5/kg (immature) (Pahlevanlo and Janardhana, 2012). Availability of enormous quantity of single mushroom needs insight for sustainable collection, preservation, transport and marketing. If such forest produce could not be sold on time, it perishes leading to financial loss as well as loss of nutritional/bioactive components. As an

alternative, such mushrooms could be preserved (e.g. freezing and drying) without major loss of their nutritional or bioactive components. Similar to termitomycetous mushrooms, several edible wild mushrooms are also available and marketed by the tribals and local dwellers in the Western Ghats and west coast of India. One such prominent edible wild mushroom is *Astraeus hygrometricus*, which is known as 'Kall-Anabe' (meaning 'stone-mushroom') (Karun and Sridhar, 2014; Pavithra *et al.*, 2015). Similar to evaluation of nutritional and bioactive components (Pavithra *et al.*, 2016, 2018), this study envisaged to assess functional properties of *A. hygrometricus* collected from the foothill region of the Western Ghats for its possible utilization in commercial food and pharmaceutical industries.

MATERIAL AND METHODS

Mushroom: Partially epigeous tender fruit bodies of gasteroid mushroom *Astraeus hygrometricus* (Pers.) Morgan were collected from five locations (50 m apart) during monsoon season (June-July 2015) in Karkala forests of the Western Ghats, India (13°12'N, 74°58'E; 65-90 m asl). Within 3-4 hr of transportation of fruit bodies to the laboratory in cool packs, they were processed. After removal of debris (soil, roots and mycelial mass), rinsed in water, drained and on slight abrasion the outermost layer of fruit bodies was removed as practiced by the tribals. Each replicate was divided into two parts and the tenderness was ascertained by white interior on cutting the fruit bodies. Those with black interior were discarded. The first part was oven dried (50-55°C) until the moisture attains below 10%. The second part was pressure-cooked with distilled water (1:1 v/v) in a pressure cooker (Deluxe stainless steel, TTK Prestige™, Prestige Ltd., Hyderabad, India; capacity, 6.5 L) and oven dried as above. After milling the dried samples, sieved (mesh # 30) and refrigerated (4°C) in air-tight glass containers for assessment of functional properties.

Protein solubility: The pH-dependant protein solubility (PS) of uncooked and cooked flours was determined according to the procedure by Were *et al.* (1997). A known quantity of flour samples (125 mg) were mixed with distilled water (25 ml) in beakers and the pH of solutions was adjusted from 2 to 10

using 1N NaOH and 1N HCl. The solutions were stirred using magnetic stirrer at 20°C (1 hr) and later centrifuged (4°C, 12000 g, 20 min). The supernatant was collected to estimate nitrogen by micro-Kjeldahl method (Humphries, 1956) and protein content was calculated ($N \times 6.25$).

$$PS (\%) = (N_s / N_f) \times 100 \quad (1)$$

(where N_s , nitrogen content in supernatant; N_f , nitrogen content in the mushroom flour sample).

Gelation property: The protocol proposed by Coffman and Garcia (1977) was employed to determine the least gelation concentration (LGC) of uncooked and cooked mushroom flours. The suspension of flour samples in distilled water (10 ml; 2-20%) were taken in a series of test tubes. They were transferred to boiling water bath (1 hr), cooled to room temperature followed by further cooling at 4°C (2 hr). The tubes were inverted and at concentration where the sample did not slip down were considered as LGC.

Water- and oil-absorption: The water-absorption capacity (WAC) and oil-absorption capacity (OAC) were evaluated based on Beuchat (1977). A known quantity of flour (0.5 g) was mixed with distilled water (5 ml) or edible oil (Sunrich Refined Sunflower Oil, Ruchi Soya Industries Ltd., Mumbai, India) (5 ml) in graduated centrifuge tubes and vortexed (30 sec). The contents in the tubes were allowed to stand at room temperature at 30±1°C (30 min). Later the contents in tubes were centrifuged (5000 g, 30 min) and the quantity of supernatant in each tube was measured. The WAC and OAC were expressed as ml of water or ml of oil absorbed per gram flour (ml/g).

Emulsion and foam properties: The emulsion properties like emulsion activity (EA) and emulsion stability (ES) were determined based on the method described by Neto *et al.* (2001). To find out EA, mushroom flours were dispersed in distilled water (10 mg/ml; 5 ml) in centrifuge tubes and homogenised (1 min) with edible oil (5 ml) (Sunrich Refined Sunflower Oil, Ruchi Soya Industries Ltd., Mumbai, India). The emulsions were centrifuged (1100 g, 5 min) and EA was calculated.

$$EA (\%) = (H_e / H_t) \times 100 \quad (2)$$

(where H_e , height of emulsified layer in centrifuge tube; H_t , height of total content in centrifuge tube).

The ES was determined by heating the emulsion (prepared as detailed above) in the centrifuge tubes (80°C, 30 min) prior to centrifugation (1100 g, 5 min). The ES was calculated by recording the height of emulsified layer.

$$ES (\%) = (H_a / H_b) \times 100 \quad (3)$$

(where H_a , height of emulsified layer after heating; H_b , height of emulsified layer before heating).

The foam properties (FC, foam capacity; FS, foam stability) were determined using the method by Coffman and Garcia (1977). To find out FC, the mushroom flours (2 g) were mixed with distilled water (100 ml) and blend vigorously (2 min) in a blender at speed 1 (Philips HL1643, Philips India Ltd., Kolkata, India). Following formula was used for

determining foam capacity (FC).

$$FC (\%) = (V_2 - V_1) / V_1 \times 100 \quad (4)$$

(where V_2 , volume after whipping; V_1 , volume before whipping).

The FS was evaluated by observing the foam level after incubation (8 hr) at room temperature (30±1°C) and expressed as the percentage of initial foam volume.

Data analysis: The significance of functional attributes between uncooked and cooked mushroom flours was assessed by t-test using Statistica Version # 8.0 (Statsoft Inc., 2008). The relationship between functional attributes with proximal properties was assessed by principal component analysis (PCA) (SPSS 16.0:www.spss.com). The PCA plot was drawn for different functional attributes vs. proximal properties of uncooked and cooked samples separately.

RESULTS AND DISCUSSION

The functional properties of the foodstuffs are not only dependent on intrinsic factors (e.g. protein structure, size and conformations) but also dependent on many external factors (e.g. temperature and pH) (Niveditha and Sridhar, 2017). The functional properties of food materials influence the sensory features, processing and storage of food materials (Seena and Sridhar, 2005). Thus, the evaluation of functional properties of known and unknown foodstuffs assumes importance to venture into new food and nutraceutical products.

Protein solubility: Protein solubility is known to be influenced by several external factors like pH, temperature, ionic strength and other factors (freezing, heating, drying and shearing) (Vaclavik and Christian, 2003; Bolontrade *et al.*, 2013). The pH-dependent protein solubility influences other functional attributes of food systems helpful to follow the oil-water interactions (Kinsella, 1976; Niveditha and Sridhar, 2017). Except for pH 8, uncooked mushroom samples showed significantly higher protein solubility than cooked samples (Fig. 1a). At pH 10 in both samples, the protein solubility was significantly highest, which was higher in uncooked than cooked sample ($p < 0.01$). The uncooked samples showed higher protein solubility than cooked samples in acidic as well as alkaline pH compared to isoelectric pH. According to Adebawale *et al.* (2005), the balance between positive and negative charges minimises the electrostatic repulsion leading to reduction of protein solubility in isoelectric pH. This feature of *A. hygrometricus* will be useful in food industry in preparation of acid- or alkaline- based foodstuffs especially protein-rich carbonated beverages (Fasuyi and Aletor, 2005). In addition, uncooked mushroom with highest solubility at pH 10 is helpful in formulation of infant food products (Idouraine *et al.*, 1997).

Gelation property: The gel formation occurs due the denaturation of native protein in food samples. This is one of the important properties of raw material to modify the food texture. It depends upon several factors like water content, protein concentration, ionic strength, time, temperature and pH of the test samples (Raikos *et al.*, 2007). The least gelation concentration (LGC) was higher for cooked than uncooked

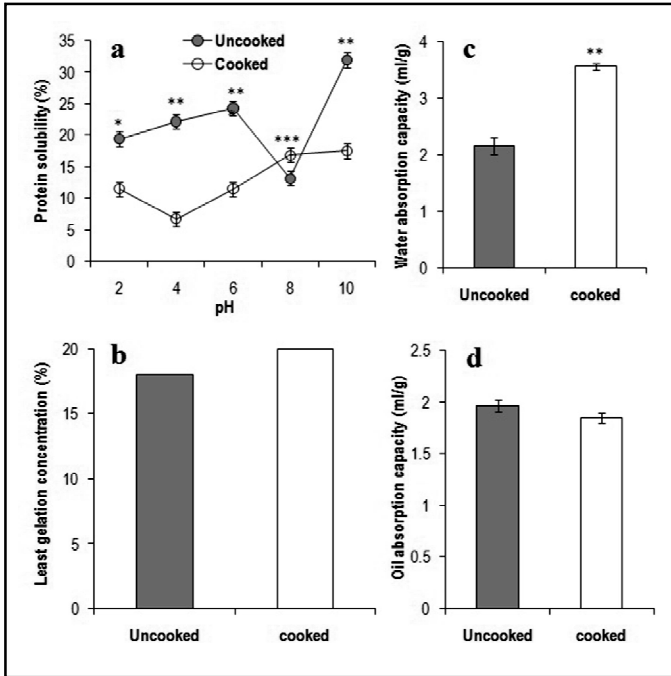


Fig. 1 Protein solubility (a), least gelation concentration (b), water-absorption capacity (c) and oil-absorption capacity (d) of uncooked and cooked tender fruit bodies of *Astraeus hygrometricus* (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

mushroom samples (20 vs. 18%) (**Fig. 1b**). The LGC of *A. hygrometricus* is higher compared to other macrofungi like *Ganoderma* spp. (14%), *Hebeloma mesophaeum* (12%) and *Omphalotus olearius* (12%) found in Nigeria (Aremu *et al.*, 2009). Interestingly, the LGC of *A. hygrometricus* is on par with wild legume seeds (*Canavalia cathartica* and *C. maritima*) of coastal sand dunes of southwest India (Seena and Sridhar, 2005). The gelling provides structural matrix for holding water, flavours, sugars and other ingredients which is useful in development of new products (Aremu *et al.*, 2006; Appiah *et al.*, 2011). Gelling will also be useful as binding agent to provide good consistency for semi-solid food formulations (Shad *et al.*, 2011). The gelling property of uncooked and cooked *A. hygrometricus* provide scope for developing food items especially puddings and snacks needs thickening or gelling.

Water- and oil-absorption: The water absorption capacity (WAC) of mushroom flour was significantly higher in cooked than uncooked samples ($p < 0.01$) (**Fig. 1c**), while it was opposite for the oil-absorption capacity (OAC) (**Fig. 1d**). The lower OAC compared WAC depicts the nutritional benefit of *A. hygrometricus*. Compared to other macrofungi, the WAC of uncooked *A. hygrometricus* is lower than *Ganoderma* spp., *H. mesophaeum* and *O. olearius* (2.2 vs. 2.6-3.9 ml/g), while the WAC of cooked *A. hygrometricus* is higher than *H. mesophaeum* (3.6 vs. 2.6 ml/g) and lower than *Ganoderma* spp. as well as *O. olearius* (3.6 vs. 3.8-3.9 ml/g) found in Nigeria (Aremu *et al.*, 2009). On the contrary, the OAC of uncooked and cooked *A. hygrometricus* is lower compared to *Ganoderma* spp., *H. mesophaeum* and *O. olearius* (1.97 and 1.85 vs. 4.5-4.8 ml/g) (Aremu *et al.*, 2009). The WAC of *A.*

hygrometricus is useful in food industries in preparation of different food products (e.g. soups, dough and baked products), while the OAC is useful in increasing the flavour as well as mouth feel of many food products (e.g. whipped toppings, sausages, desserts and sponge cakes) (Adeyeye and Aye, 1998; Chandra and Samsher, 2013).

Emulsion and foam properties: The emulsion is one of the important requirements in preparation of foodstuffs like cake, coffee whiteners and frozen desserts. The emulsion activity (EA) of *A. hygrometricus* flours ranged between 2.55 and 2.58% without significant difference between uncooked and cooked samples (**Fig. 2a**). Unlike EA, the emulsion stability (ES) was extremely high and ranged from 99-99.7% (**Fig. 2b**). The EA of *A. hygrometricus* was lower than cap, stalk and cap+stalk of *Pleurotus ostreatus* (2.6 vs. 5.3-17.3%) (Oluwafemi *et al.*, 2016), so also compared to beta-glucans derived from *Agaricus bisporus*, *Coprinus atramentarius* and *P. ostreatus* (2.6 vs. 64.3-65.5%) (Khan *et al.*, 2014). However, the ES of *A. hygrometricus* flours is comparable with beta-glucans of *A. bisporus*, *C. atramentarius* and *P. ostreatus* (99-99.7 vs. 94.6-97.7%) (Khan *et al.*, 2014). As ES of uncooked and cooked *A. hygrometricus* is extremely higher than many edible mushrooms, they are valuable to utilize for salad dressing, comminuted meat products, frozen desserts and mayonnaise (Chandra and Samsher, 2013).

The foam capacity (FC) of *A. hygrometricus* was significantly higher in cooked than in uncooked flours (12 vs. 6.1 %) ($p < 0.01$) (**Fig. 2c**), so also the foam stability (FS) (10.1 vs. 3.3 %) ($p < 0.001$) (**Fig. 2d**). The FC as well as FS of *A. hygrometricus* are lower compared to *Ganoderma applanatum*, *G. brownii*, *Ganoderma* spp., *H. mesophaeum*

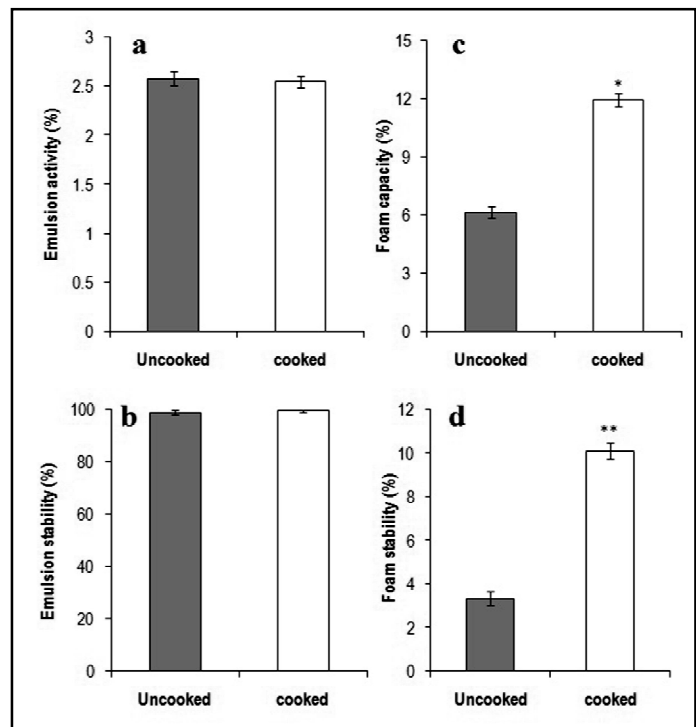


Fig. 2 Emulsion activity (a), emulsion stability (b), foam capacity (c) and foam stability (d) of uncooked and cooked tender fruit bodies of *Astraeus hygrometricus* (*, $p < 0.01$; **, $p < 0.001$).

and *O. olearius* (Aremu *et al.*, 2009; Singh *et al.*, 2014). The FC and FS of cooked samples of *A. hygrometricus* are higher compared to the beta-glucans of *A. bisporus*, *C. atramentarius* and *P. ostreatus* (Khan *et al.*, 2014). Food formulations like frozen desserts, cakes, whipped toppings and ice-cream mixes demands high FC and FS, cooked samples of *A. hygrometricus* could be used in such food stuffs (Niveditha and Sridhar, 2017).

Functional vs. proximal attributes: The principal component analysis (PCA) was carried out between four proximal components of *A. hygrometricus* (crude protein, Cp; total lipids, Tl; crude fibre, Cf; total carbohydrates, Cb) against seven functional attributes [pH-dependant protein solubility, Ps (data of the highest solubility at pH 10 was considered), water-absorption capacity, Wa; oil-absorption capacity, Oa; emulsion activity, Ea; emulsion stability, Es; foam capacity, Fc; foam stability, Fs]. Comparison of proximal qualities vs. functional properties was carried out separately by suffixing U for uncooked and C for cooked mushroom samples. In uncooked samples, the PCA showed two components with 100% variance (Eigen value, <1; PC1, 62.9%; PC2, 37.1%) with only one minor cluster. The total lipids (TlU) was associated with oil-absorption capacity (OaU) as well as emulsion stability (EsU) (**Fig. 3a**). The PCA for cooked samples resulted in two components with 100% variance (Eigen value, <1; PC1, 65.3%; PC2, 34.7%) with one each of major and minor clusters. In the major cluster, crude fibre (CfC) was associated with pH-dependant protein solubility (PsC), oil-absorption capacity (OaC), emulsion activity (EaC) and emulsion stability (EsC), while in the minor cluster the total lipids (TlC) associated with only foam capacity (FcC) (**Fig. 3b**).

This study provided evidence that the proximal qualities vs. functional features of uncooked and cooked *A. hygrometricus* differs drastically. The total lipids in uncooked samples seems to control two functional properties (oil-absorption and emulsion stability), which has been supported by significantly higher quantity of total lipids in uncooked than cooked samples (Pavithra *et al.*, 2018). In cooked samples, the crude fibre content alone seems to have control over four functional properties (pH-dependent protein solubility, oil-absorption capacity, emulsion activity and emulsion stability) justified by significant increase in crude fibre on cooking *A. hygrometricus* (Pavithra *et al.*, 2018). In spite of high content of total carbohydrates in *A. hygrometricus*, it has not been associated with any functional attributes studied.

CONCLUSIONS

Uncooked and cooked tender *A. hygrometricus* is known for good nutritional qualities (e.g. protein, fibre, carbohydrates, essential minerals, essential amino acids and carotenoids) as well as bioactive properties (antioxidant and radical-scavenging activities) (Pavithra *et al.*, 2016, 2018). This study added additional information on functional properties of tender *A. hygrometricus* like increased protein solubility at acidic as well as alkaline pH. There was a narrow difference between uncooked and cooked mushroom in least gelation concentration, oil-absorption capacity, emulsion activity and emulsion stability. Cooked mushroom showed good water-

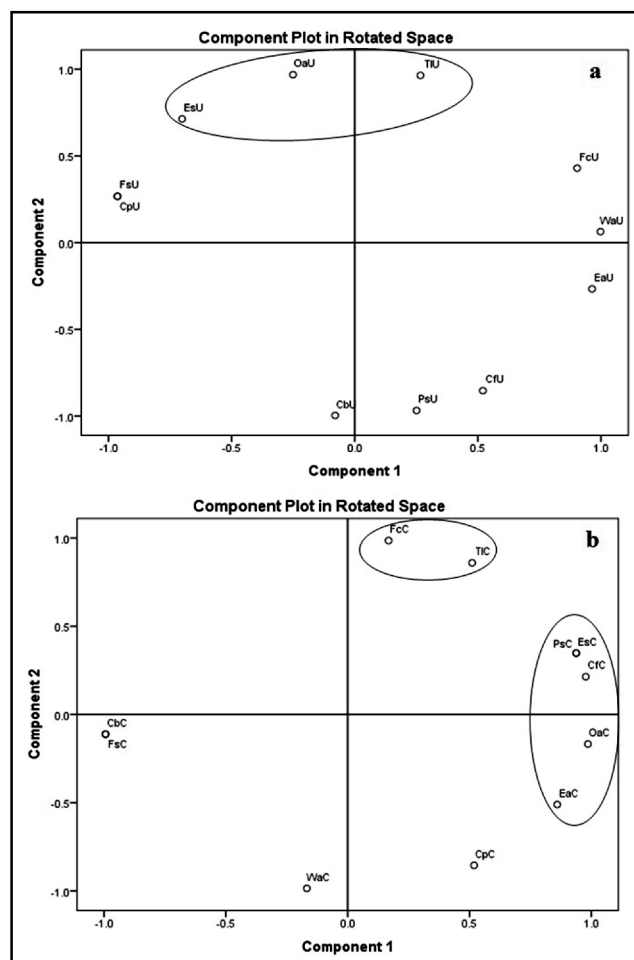


Fig. 3 Principal component analysis of uncooked (with suffix U) (a) and cooked (with suffix C) (b) tender fruit bodies of *Astraeus hygrometricus* between proximal components (crude protein, Cp; total lipids, Tl; crude fibre, Cf; total carbohydrates, Cb) and functional properties (pH-dependant protein solubility, Ps; water-absorption capacity, Wa; oil-absorption capacity, Oa; emulsion activity, Ea; emulsion stability, Es; foam capacity, Fc; foam stability, Fs).

absorption capacity, foam capacity and foam stability. The total lipids and crude fibre in tender *A. hygrometricus* have major role in controlling functional attributes. Thus, uncooked as well as cooked tender *A. hygrometricus* serve as a good raw material in production of functional foods and pharmaceuticals with desired characteristics.

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