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Study of antioxidant properties of chickpea and oat flour meat analogue containing beetroot extract

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Abstract
This research was aimed in order to find the potential of chickpeas as for the preparation of healthy meat analogue. Meat analogues were prepared in three different ratios (60:20), (50:30), (40:40) of chickpea and oat flour combinations. Beetroot extract was added in order to study its antioxidant properties. Sensory evaluation showed that meat analogue with 50:30 proportion of chickpea and oat flour was mostly accepted in terms of appearance, taste, texture, color, flavor and overall acceptability. Nutritional evaluation of the optimum product showed significant amount of essential nutrients. Increased antioxidant activity was found which can be attributed due to the addition of beetroot extracts. Thus, it was concluded that chickpea can be used to develop healthy meat analogues without compromising protein and micronutrient consumption.

Keywords: Meat analogue, chickpea, high antioxidant activity, beetroot extract, vegetarian, dietary fibre

1. Introduction

Meat is by far the first choice of food for people when it comes to their diet. Humans have considered meat to be an essential constituent of their diet and it played a very fundamental role in the human evolution as it has been linked to the brain growth and development of human beings (He *et al.* 2020) [9]. This is due to the distinct taste and flavor it imparts and also the benefits it provides in the nutritional aspect. Meat is considered a powerhouse of high value proteins, variety of fats including omega-3 polyunsaturated fatty acids, zinc, iron, selenium, potassium, magnesium, sodium, vitamin A, B-complex vitamins and folic acid (Ahmad *et al.* 2018). However, it has tagged with a number of negative impressions since the beginning ranging from ritualistic and environmental concerns to health and welfare issues (van der Weele *et al.* 2019) [34]. The need for natural resources for rearing meat animals in the form of water, feed, fodder etc. has led to increase in the exploitation of resources like land, water etc. Religious concerns like the public's preference to halal and safely processed foods also added to the disadvantages of meat consumption (Kumar *et al.* 2016) [18]. It has also been found that the intake of red meat has been found to cause ischemic heart disease, obesity, worsening of joint inflammation and colorectal cancer (Sun *et al.* 2020) [32]. These factors led to consumers in search of alternative products which provided them with all the nutritional qualities of meat products.

Meat analogue is a food commodity that has the cosmetic, chemical, and nutritional qualities of certain types of meat (Malav *et al.* 2013) [22]. Recent years saw a high increase in the development of new sustainable meat substitutes to reduce the negative environmental impact of industrial-scale meat production for human consumption (Joshi & Kumar, 2015) [14]. Meat alternative foods have long been used since ancient times like the centuries old recipes of wheat gluten, rice, mushrooms, legumes, pulses, tempeh, tofu etc. mixed with seasonings and flavors to make it taste like meat (Kyriakopoulou *et al.* 2019) [20]. The recent commercialized usage of texturized vegetable proteins (TVP) has paved way for new products which provided texture and taste properties of meat (Ismail *et al.* 2020). These properties make them the ideal products for diet conscious people who want to have the experience of eating meat but also be devoid of its negative impact. These foods are becoming more popular in the industry because of their cost advantages, comparatively stable price due to them being less liable to seasonal fluctuations in supply, longer shelf life and easier storage (Joshi & Kumar, 2015; Lee *et al.* 2020) [14].

Vegetarian meals currently occupy a larger than ever shelf space in today's market as a result of customers' growing health concerns and related environmental difficulties (Kumar, 2016) [18].

Many vegans choose to eat a plant-based diet because of environmental issues, ethical concerns about animal welfare, the use of antibiotics and growth hormones in animal production, the threat of animal-borne diseases, and the health benefits of eating plants (Craig, 2009) [5]. Studies have found that plant protein based diets has made a significant impact in the reduction of body weight, cholesterol, blood pressure levels which in turn reduced the risk of stroke, heart disease and cancer (He *et al.* 2020) [9]. Depending on the protein source used, meat analogues have been demonstrated to have a diversity of proteins. Vegetarian diets can be supplemented with proteins derived from legumes, nuts, seeds, and whole grains. As a result, a well-designed vegetarian diet should provide 12.5 percent protein-derived energy on average (Pilis *et al.* 2014) [25].

Soy protein, wheat gluten, and pea protein are three of the most common protein ingredients used in the production of meat substitutes (He *et al.* 2020) [9]. Because of its abundance, low cost, meat-like texture after hydration, and high-quality amino acid composition, soy protein, including soy protein isolate and soy protein concentrate, is the most common ingredient in structured plant protein products. Soy protein may also aid in the prevention of cardiovascular disease (Sun *et al.* 2020) [32]. The use of other protein sources like chickpeas have been very rarely attempted. Chickpeas are high in protein and carbs and are a healthy source of both. Several studies have detailed the physicochemical and nutritional properties of chickpeas, revealing that they can be used to replace meat in products such as nuggets and sausages (Verma *et al.* 2012) [35]. Chickpea globulins and albumins, like those found in other legumes, are the two primary fractions found in beans (Singh *et al.* 2008) [30]. Albumins are crucial in chickpea beans because they contain the majority of metabolically vital enzymes and proteins. Because of their excellent biological value, high biodisponibility, well-balanced amino acid content, and low antinutritional components, chickpea proteins are more appreciated than proteins from other legumes (Kaur and Singh, 2007) [15]. The low fat content of chickpea beans, combined with their unique properties, justifies the current interest in obtaining chickpea protein isolates and concentrates, as well as their functional characterization (Aurelia *et al.* 2009) [1]. Chickpea proteins show a high regard of functional properties such as solubility, water absorption capacity, emulsifying, foaming and gelling (Grasso *et al.* 2022) [7]. Chickpea flour has a protein content of 17-21 percent, a fat content of 5-7 percent, a carbohydrate content of 61-62 percent, 3 percent ash, and a water content of 9-12 percent (Boye *et al.* 2010) [2]. Chickpeas have indeed been processed into flour in order to improve its food application functionality.

The chemical makeup of oat grain is particularly useful, and the combination of nutritional substances inherent in this raw material makes it a profitable component of human diet (Sadiq Butt *et al.* 2008) [28]. Oat grain provides protein with a healthy amino acid profile, a beneficial fatty acid profile, a high level of PUFA, and a big number of water soluble beta-glucans and antioxidants (Gambus *et al.* 2011). Globulins make up the majority of oat proteins (about 80% of total protein), with prolamins and glutenins accounting for the remaining 20%. (Sontag-Strohm et al. 2008) [31]. Numerous studies have found that oat-rich diets can help with inflammatory bowel illness and celiac disease (Thies *et al.* 2014) [33], CVD progression (Ruxton & Derbyshire., 2008) [27], and glucose control in type 2 diabetes (Hou *et al.* 2015) [12]. Soluble fibre (β-glucan) found in oats and oat-based products has been linked to a variety of health benefits. In the food sector, soluble β-glucan and other bioactive compounds from cereals like barley or oats, as well as their fractions, have been demonstrated to have potential nutritional benefits (Tiwari *et al.* 2013). Many clinical investigations have found a link between oat β-glucan consumption and lower blood cholesterol and glucose levels (Ruxton and Derbyshire, 2008; Hlebowicz *et al.* 2008) [27, 10].

Antioxidants are utilized in meat and meat products to reduce oxidative changes. Oxidative alterations can degrade the quality of meat and meat products by altering their sensory and nutritional qualities. Beetroot (*Beta vulgaris* L.) has recently gained popularity as a potential "functional food" in the matter of healthy diet (Chen *et al.* 2021) [3]. Despite the fact that beetroot has long been a staple of European cuisines, knowledge of its nutritional worth is extremely restricted. Consumers now have a better understanding of beetroot's biological activity because to the development of preclinical experiments. Several recent studies have suggested that betalains may lower the incidence of some cancers, cardiovascular and cerebrovascular illnesses, as well as liver and kidney damage (Kavitha *et al.* 2013). Beetroot has a lot of nitrate, which has a lot of nutritious value. Many people use fresh beetroot juice orally to supplement nitrate and so improve physiological reactions and minimize the risk of cardiovascular and cerebrovascular diseases (Webb *et al.* 2008) [36]. When meat analogues are sold as a replacement for raw meat products, they must undergo colour changes during cooking. As a result, heat stable colourants, even those of natural origin, must be substituted with or blended with colourants that allow for a colour change akin to that of meat when cooked or fried. The denaturation of myoglobin causes the colour of meat to change from red to pink or grey-brown at temperatures around 75 degrees Celsius (Hollenbeck *et al.* 2019) [11]. Betanin and beetroot extracts are recommended as additives that attribute a raw meat colour and experience colour changes owing to thermal degradation to simulate this (Kyed and Rusconi, 2009) [19]. Beyond Meat used beetroot extract as a colourant in their "raw" burger composition. Furthermore, bleeding veggie burgers use beetroot juice to provide the sensation of juiciness while also giving the product a distinct meat colour. Therefore taking into consideration of all the benefits provided by these ingredients, the present study was framed with the objective of preparing a meat analogue from chickpea and oat flour with enhanced antioxidant properties.

2. Materials and Methods

2.1 Procurement of raw materials

The required materials were all procured from various sources. Chickpeas, oat flour and beetroot extract were purchased from the local market around Phagwara, Punjab. Seasonings and spices used were salt, nutritional yeast, chicken masala, garlic powder, onion powder, chili powder, turmeric powder, black pepper powder, and vegetable oil for fat source. The spices were acquired from the food laboratory of Lovely Professional University.

2.2 Preparation of chickpea flour

The chickpeas were first germinated in order to make the chickpea flour. For germination, the chickpeas were first soaked in sodium hypochloride solution for 30 minutes and then later soaked in water for 12 hours. The soaked chickpeas were then later kept for germination by wrapping them in cloth and keeping in a cool and dark place for 48 hours. The chickpeas were kept moist by sprinkling water at regular intervals to ensure that it doesn't get dried up. The germinated chickpeas were then further dried in hot air oven and then later grinded into a fine powder.

2.3 Preparation of meat analogue

The meat analogues were prepared in three different ratios (60:20, 50:30 and 40:40) of 50 g each in order to find the product with the optimum ratio. The different ratios were achieved by changing the ratios of the chickpea and the oat flour. First, the chickpea flour and oat flour were added according to the formulation and mixed in a bowl. Spices and the rest of the ingredients were then added later followed by beetroot extract. Then vegetable oil was added and the mixture was mixed thoroughly. The mixture was then kneaded with adequate amount of water to ensure good mixing of the ingredients and for the formation of the dough. The dough was then sized into the size of patties and rounded into a ball shape followed by flattening them into shapes that resembles a burger patty. They were then placed on an oiled baking tray and rested for a few minutes. The patties were precooked by steaming. The patties were arranged overlapping one other, and grease paper was placed between each patty to facilitate separation prior to cooking. The patties were then stored in a freezer. The patties were then later cooked in a pan and served in three different plates for sensory evaluation.

2.4 Sensory Evaluation

Three different ratios of meat analogues were prepared for sensory evaluation by using the nine-point hedonic scale (1= disliked extremely, 9= liked extremely). Each meat analogue was cooked and presented in a randomized order. The sensory panelist comprised of a total of 6 teachers and 4 students from the School of Agriculture, Lovely Professional University, Punjab, India. The products were assessed on appearance, taste, colour, flavour, texture, and overall acceptability.

2.5 Nutritional Composition

The methodologies employed for every sample composition analysis were based on those provided by AOAC [21]. The nutritional composition of the optimum ratio of the meat analogue, comprising carbohydrate, protein, fat, and total dietary fibre, was used to compare with commercial meat analogue. The moisture content of the prepared meat analogue was determined using the oven-drying method after drying the sample at 105°C for 4 hours in an air-drying oven. The sample's weight was tested at regular intervals, and the consecutive weight was recorded. Ash content of the meat analogue was determined by the method of dry ashing which involved the usage of a muffle furnace at 550°C for 6 hours. The crude fat content was determined using Soxhlet extraction method by the soxhlet apparatus. The Kjeldahl technique and the Kjeldahl nitrogen analyzer were used to determine crude protein content. The prepared meat analogue's total carbohydrate content was estimated using the equation:

Total carbohydrate (%) = 100% − (%MC + %F + %P + %A + %CF),

Where; MC = moisture content, F = fat, P = protein, A = ash and CF = crude fibre

The antioxidant activity of the prepared meat analogue was analysed using DPPH test. The DPPH reagent was prepared and 1 g of sample is dissolved with 10 ml of ethanol and centrifuged at 6000rpm for 30 minutes. Absorbance is read at 525nm for calculating the DPPH free radical. The DPPH inhibition percentage was thus calculated.

3. Results and Discussion

3.1 Sensory evaluation

By the end of the research two ratios of meat analogue, sample 1 (40:40) and sample 2 (50:30) were selected for sensory evaluation. The ratio of 60:20 was eventually discarded since it gave a product with a hard texture and poor taste. Sensory attributes were evaluated and the results are presented in table 1.

The inclusion of varied ratios of chickpea and oat flour had a significant effect on the appearance, taste, color, texture, flavor, and overall acceptability of the manufactured meat analogue samples, according to the findings. The results showed that the sample 2 with the 50:30 ratio had the highest overall acceptability and was well received among the panelists. In terms of appearance, there was no significant difference between the two samples. However, major difference was found in other attributes and was highly in favor of sample 2. A graphical representation of the sensory evaluation of the prepared meat analogue is shown in fig. 1.

Taste is a very crucial characteristic for consumers when choosing a food product. The sample 2 had the highest level of taste acceptance. Based on all attributes, sample 2 was most preferred by panelists and was considered the optimum ratio. Sample 2 was then selected for further tests and proximate analysis.

Table 1: Sensory characteristics of meat analogue patties with different ratios

Characteristics	Sample 1	Sample 2
Appearance	7 ± 0.63	7.4 ± 0.66
Colour	6.6 ± 0.48	7.2 ± 0.4
Texture	6.2 ± 0.6	7.4 ± 0.91
Flavour	6.5 ± 0.8	7.4 ± 0.66
Taste	6.7 ± 0.9	7.3 ± 0.64
Overall Acceptability	6.5 ± 0.5	7.6 ± 0.66

Values are mean ± S.D

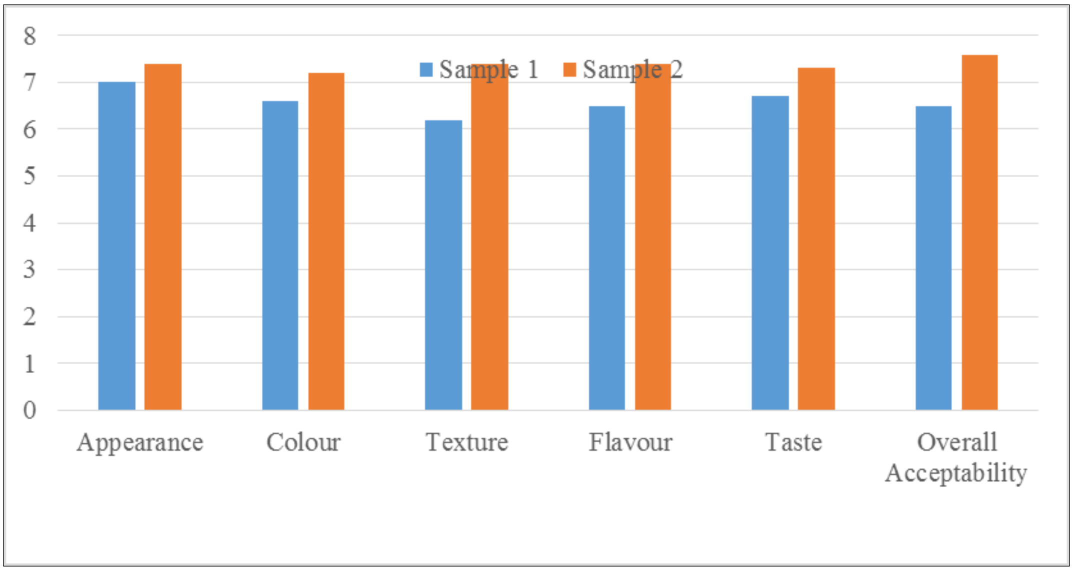

Fig 1: Graphical representation of sensory attributes of chickpea and oat flour meat analogue

3.2 Nutritional evaluation of highly acceptable chickpea and oat flour meat analogue

The most acceptable meat analogue with 50:30 ratio of chickpea and oat flour combination was chemically analyzed for proximate composition and total antioxidant activity with the help of standardized methods and shown in table 2. They are then compared with the meat analogues prepared from other studies and researches.

Table 2: Nutritional composition of chickpea and oat flour meat analogue

Nutritional Composition	Composition
Moisture (%)	43.87 ± 0.66
Protein (%)	16.57 ± 1.56
Carbohydrate (%)	26.65 ± 1.99
Fat (%)	4.59 ± 0.08
Crude Fibre (%)	4.58 ± 0.06
Ash (%)	3.73 ± 0.25
Energy (cal/g)	4950.87 ± 227.53
Antioxidant Activity (%)	23.17 ± 4.204

Values are mean ± S.D

It was found that meat analogue made from chickpea and oat flour had a protein contein of 16.57% which can be considered as average content of protein. It was slightly more than the protein content of the commercial meat analogue compared by Hamid *et al.* (2020) [8] (14.26%) but slightly lower than the meat analogue from jackfruit by-product (20.67%). It was also higher than the imitation nuggets made from chickpea and TVP by Faujan *et al.* (2018) and meat analogue nuggets from mushroom by Sharma *et al.* (2011). It was also significantly higher than the meat analogue made from wheat gluten by Kumar et al (2014). However, it was comparatively lower against meat analogues made from other legumes such as peas, soya etc. Meat analogues made from pea proteins by De-Angelis *et al.* (2020) [6] had a protein content of 55.59%. However, according to their previous investigations, the protein content of imitative burgers ranged from 17.7 to 25.5g which is slightly close to our product. Meat analogues prepared from Mucuna bean seed flour by Omohimi *et al.* 2014 [23] had a protein content of 31.29%. Peanut-based meat analogue prepared by Rehrah *et al.* (2009) [26] had a protein content of 31.70% while the commercial soya based meat analogue that they compared with had a protein content of 29.42%.

Carbohydrate content of every meat analogue can vary according to the ingredients present in it. Chickpea and oat flour meat analogue had a relatively higher amount of carbohydrate (26.65%) when compared to meat analogues by Hamid *et al.* (2020) [8] and the commercial meat analogues that they compared their product with and De- Angelis *et al.* (2020) [6]. Chickpea and oat flour meat analogue had a fat content of 4.59% which was almost on par with products from studies of Faujan *et al.* (2018), De-Angelis *et al.* (2020) [6]. It was slightly lower compared to studies from Hamid *et al.* (2020) [8], Sharma *et al.* (2011) and Rehrah *et al.* (2009) [26]. The meat analogue had an ash content of 3.76% which was almost in range with other products of the aforementioned studies. The prepared meat analogue had a fibre content of 4.58% which was slightly more than the meat analogue studied by Hamid *et al.* (2020) [8] and significantly higher than the commercial meat analogue that they compared with (1.67%). Meat analogues prepared by Omohimi *et al.* (2014) [23] had a similarly very low crude fibre content (1.98%).

The moisture content of samples is thought to be one of the most critical factors determining shelf stability. When opposed to lower moisture products, high moisture products typically have a shorter shelf-life stability. Chickpea and oat flour meat analogue had a moisture content of 43.87% which was of the same range when compared to products from Faujan *et al.* (2018), Chiang *et al.* (2018), Hamid *et al.* (2020) [8]. However, meat analogues studied by Omohimi *et al.* (2014) [23] had a very less moisture content of 9.68%. According to the findings, chickpea and oat flour was found to have energy content of 4950.87 cal/g. Meat analogues made from jackfruit by-product by Hamid *et al.* (2020) [8] had an energy content of 271.65 kcal/g while commercial meat analogue that they compared with had energy value of 265.65 kcal/g. DPPH assay tests conducted showed that chickpea and oat flour meat analogue had DPPH inhibition % of 23.17. Antioxidant activity of crab meat analogue prepared by Jin *et al.* (2016) [13] showed a maximum amount of 7.2% on the first week of storage which later got drastically reduced. The increased antioxidant activity in chickpea and oat flour meat analogue can be attributed to the addition of beetroot extracts in it which might have enhanced it’s enhanced its antioxidant potential. Further antioxidants present in chickpea flour and oats flour have also helped in the increased antioxidant activity in the meat analogue.

4. Conclusion

The concept of meat analogues and their prominence are growing, which can be traced to consumers' growing concern about their health and interest in nutritional food diets. Meat analogues can be considered a viable replacement for meat and meat products in terms of nutrition, health, and benefits. It can be concluded from the above research that chickpea and oat flour can be a suitable combination for the development of meat analogues. Sensory evaluation of the prepared meat analogue showed that the sample with 50:30 ratio of chickpea and oat flour was mostly accepted and preferred by consumers. Further analysis of the optimum ratio for nutritional composition showed that they contain a significant amount of all the essential nutrients. The addition of beetroot extract was done to enhance the antioxidant potential of the prepared meat analogue. The research can be concluded to the fact that chickpeas can be considered as suitable replacement for meat in a diet. Even though, the prospect of usage of chickpeas as a suitable source of protein in meat analogue looks promising, further research is required on study of its antioxidant potential and thereby the scope of such meat analogues as a nutraceutical.

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