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RESEARCH ARTICLE
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Process Optimization of Assorted Nutraceutical Biscuits for Improved Protein Accessibility

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ABSTRACT

Background: Assortment of Cereal-legume flour has been gaining attention nowadays for being a promising meat analogue for vegetarian population. Biscuits containing defatted soya flour (DSF), wheat flour (WF) and nutraceutical ingredients were developed for improved protein quality.

Methods: T_1, T_2 and T_3 were standardized at 60, 50 and 40% DSF levels to WF against T0 (100% WF). T_3 was finalized for assorted nutraceutical biscuits (T_4 and T_5) and half portion of DSF was replaced with nutraceutical ingredients. Treatments and raw ingredients were analyzed for trypsin inhibitor, amino acid content and *in vitro* protein digestibility.

Result: T_4 was the highly acceptable treatment. A significantly ($p<0.05$) higher lysine and methionine contents were found in assorted nutraceutical biscuits than the control samples. *In vitro* protein digestibility was also higher in T_4 and T_5. Diverse nutraceuticals can be used in assortment of value added biscuits to obtain quality protein characteristics.

Key words: Assorted nutraceutical biscuits, Chemical characteristics, Process optimization, Sensory evaluation.

INTRODUCTION

Biscuits have good potential for nutritional improvements, since this food product can be supplemented with diverse functional ingredients to target special health conditions (Nogueira and Steel, 2018). Besides, biscuits are globally consumed snacks, attributed to their longer shelf life and variation in sensory aspect, hence production of different types of biscuit has been increasing during contemporary times (Canalis *et al.,* 2017). Further, nutritive value of biscuits can be enhanced through various supplementation and fortification techniques considering age-group specific requirements or to achieve therapeutic targets during disease conditions (Davidson, 2019). Copious interventions to check the efficacy of food by-products incorporated biscuits in view of consumer acceptance and disease prevention have been carried out (Romeo *et al.,* 2020).

Refined WF is main ingredient for biscuit production but lacks in dietary fiber, amino acid etc. Till now, numerous cereal-pulse flour blends based biscuits are developed and evaluated by various researchers and wheat soya based biscuits are found suitable because of their cost effectiveness and enhanced nutritional profile; thereby, recommended to obtain the therapeutic aspect. In addition, composite flour mixture developed from legume and cereal flours has increased protein content with improved amino acid composition, because of lysine content present in legumes (Cappa *et al.,* 2020). Among various legumes, soyabean (Glycine max) is abundantly produced and consumed in Asian households. It is rich in protein and fiber, along with health promoting substances such as isoflavones (Acharya, 2020). DSF can be more viable in biscuit production with high protein digestibility, amino acid content and varying protein dispersibility index (PDI) *i.e.* degree of protein denaturation during heat treatment (Hernandez and Serna-Saldivar, 2019).

Nutraceuticals are helpful in enhancing food value with both special physical and psychological conditions (Chintale *et al.,* 2013). As being natural, these compounds have lesser side-effects as compared to traditional and modern medications. Nutraceuticals can be classified as nutrients, herbs or botanical extracts and dietary supplements (Sapkale *et al.,* 2012). Nutraceuticals possess bioactive components which potentially promote health and well-being by reducing the progression of diseases (Aryee, 2015). Likewise, incorporation of both

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whole fruits and vegetables and their by-products (apple pomace powder, mango peel, orange peel *etc.*) as functional and bioactive ingredients become a latest trend in the food industry targeting the increased health knowledge and concern among consumers (Pakhare *et al.,* 2018). Numerous scientists scrutinized the effect of bioactive compounds pre sent in various plant based foods and herbal nutraceuticals such as soy (Krishna Moorthy and Venugopal, 2008), tomato (Wei and Giovannucci, 2012; Zu *et al.,* 2014), ginger (Rashid and Umamaheswari, 2017; Karna *et al.,* 2012; Brahmbhatt *et al.,* 2013), citrus peels (Rawson *et al.,* 2014; Rafiq *et al.,* 2018; Wang *et al.,* 2014) and curcumin (Cimino *et al.,* 2012) for their potential for therapeutic targets. Thus, assorted nutraceutical biscuits using whole WF and DSF as composite flour supplemented with fruit and vegetable components have been developed to improve amino acid content and *in vitro* protein digestibility.

MATERIALS AND METHODS

Process optimization of assorted nutraceutical biscuits

Five different treatments of biscuit (three control and two assorted nutraceutical treatments) were developed at Food Technology Laboratory, Lovely Professional University, Phagwara during the year 2018-19 (Table 1). Traditional creamery method by Meena and Meena (2004) was practiced. Dough was refrigerated and after 45 minutes proofing, it was kneaded back and spread to a 2.5 mm thick sheet; the sheet was cut into different shapes and baked at 180°C for 12 minutes.

Evaluation of biscuits

Sensory evaluatio

Sensory evaluation was carried out with a panel of 10 semi-trained judges using 9 point hedonic scale. Scores obtained for each treatment were recorded.

Chemical analysis

Both ingredients and biscuits were analysed for their trypsin inhibitor (Roy and Rao, 1971), amino acid composition i.e. lysine (Carpenter, 1960) and methionine (Horn *et al.,* 1946) estimation and *in vitro* protein digestibility (Akeson and Stachman's, 1964), in triplicates, on wet weight basis to determine the nutritional quality of developed products.

Statistical analysis

The recorded data was subjected to Tukey's test, One-way ANOVA (analysis of variance) using GraphPad Prism software version 5.01. Mean difference between the treatments was analysed at the 5% level of significance.

RESULTS AND DISCUSSION

Sensory characteristics of biscuits

Sensory characteristics are the most important attributes of a food product for judging the quality of food products and consumer's preferences in terms of its level of acceptability. In present study, significant difference ($p<0.05$) has been observed in appearance, colour, texture and flavour among biscuit samples of three control and two treatments (Table 2). Dark brown colour of T_5 could be, due to degradation of carotenoid pigments and maillard reaction under which non-enzymatic browning takes place during baking (Imeneo *et al.,* 2021). Dark colour and bitter taste were the factors for its lesser overall acceptability scores. Zaker *et al.* (2012) too reported a drastic reduction in appearance, colour, texture and flavour with every 10% increase of soy flour in the basic ratio of flour mix which could be due to heat liable browning and beany flavour of baked products containing higher protein. In the pre sent study, scores obtained for appearance, colour and texture in T_5 were in agreement with the figures reported by Zaker *et al.* (2017). Lower proportion of defatted soya flour blended

Table 1: Treatments for formulation of biscuits by incorporating other food ingredients.

Ingredients	T_0 (g)	T_1 (g)	T_2 (g)	T_3 (g)	T_4 (g)	T_5 (g)
Defatted soya flour	-	150	125	100	50	50
Wheat flour	250	100	125	150	150	150
Butter	125	125	125	125	125	125
Sugar (Powdered)	125	125	125	125	125	125
Ammonium bi-carbonate	3.5	3.5	3.5	3.5	3.5	3.5
Ginger (Powder)	-	-	-	-	5	-
Wheat bran	-	-	-	-	17.5	17.5
Tomato pulp	-	-	-	-	25	25
Tangerine peel (Powder)	-	-	-	-	-	5
Black pepper	-	-	-	-	1.25	1.25
Turmeric	-	-	-	-	1.25	1.25

Where, T_0 = 100% Wheat flour; T_1 = 60% Defatted soya flour and 40% wheat flour; T_2 = 50% Defatted soya flour and 50% Wheat flour; T_3 = 40% Defatted soya flour and 60% Wheat flour; T_4 = 20% Defatted soya flour, 60% Wheat flour, 2% Ginger (Powder); 7 % Wheat bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper; T_5= 20% Defatted soya Flour, 60% Wheat flour, 2% Tangerine peel (Powder); 7% Wheat bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper.

Table 2: Sensory evaluation of control and assorted nutraceutical biscuits.

Parameters	T_0	T_1	T_2	T_3	T_4	T_5
Appearance	8.4±0.42^a	7.1±0.39^d	7.7±0.48^c	8.3±0.48^b	8.4±0.46^a	7.3±0.48^b
Color	8.5±0.34^a	6.9±0.32^d	7.55±0.44^c	8.35±0.53^b	8.3±0.42^a	7.3±0.42^b
Texture	8.5±0.36^a	6.8±0.63^d	7.55±0.68^c	8.4±0.66^b	8.2±0.63^a	7.0±0.82^b
Flavor (aroma/taste)	8.5±0.60^a	6.4±0.70^d	7.3±0.67^c	8.4±0.70^b	8.0±0.53^a	6.6±0.52^b
O. A.	8.4±0.23^a	6.65±0.47^d	7.4±0.57^c	8.3±0.63^b	8.25±0.42^a	7.0±0.53^b

*Values are Mean±SD from ten determinations; Different superscripts in the same row are significantly different ($p<0.05$).
Where, T_0 = 100% wheat flour; T_1 = 60% Defatted soya flour and 40% wheat flour; T_2 = 50% Defatted soya flour and 50% wheat flour; T_3 = 40% Defatted soya flour and 60% wheat flour; T_4 = 20% Defatted soya flour, 60% Wheat flour, 2% Ginger (Powder); 7% Wheat bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper; T_5= 20% Defatted soya flour, 60% Wheat flour, 2% Tangerine peel (Powder), 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper; O. A.= Overall acceptability.

Table 3: Trypsin inhibitor and amino acid content of raw ingredients and biscuits.

	Trypsin inhibitor (mg/g)	Lysine (g/100 g protein)	Methionine (g/100 g protein)
Ingredient samples			
Defatted soya flour	4.7±0.4^a	3.71±0.22^a	0.71±0.05^c
Wheat flour	0.0±0.0	1.06±0.05^d	0.83±0.02^b
Wheat bran	0.0±0.0	3.64±0.21^b	0.97±0.05a
Tomato	0.0±0.0	1.44±0.12^c	0.42±0.08^d
Tangerine peel	0.0±0.0	0.0±0.0	0.0±0.0
Ginger	0.0±0.0	0.0±0.0	0.0±0.0
Biscuit samples			
T_0	0.0±0.0	1.04±0.05^f	0.77±0.03^f
T_1	0.47±0.05^a	2.43±0.12^c	1.61±0.15c
T_2	0.39±0.4^b	2.21±0.13^d	1.41±0.26^d
T_3	0.36±0.2^c	1.98±0.19^e	1.27±0.23^e
T_4	0.33±0.2^e	4.24±0.14^a	2.18±0.33^a
T_5	0.34±0.3^d	4.01±0.15^b	2.07±0.5^b

*Values are Mean±SD from triplicate determinations; Different superscripts in the same column are significantly different ($p<0.05$).
Where, T_0 = 100% Wheat Flour; T_1 = 60% Defatted Soya Flour and 40% Wheat Flour; T_2 = 50% Defatted Soya Flour and 50% Wheat Flour; T_3 = 40% Defatted Soya Flour and 60% Wheat Flour; T_4 = 20% Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder), 7 % Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper; T_5 = 20% Defatted Soya Flour, 60% Wheat Flour, 2% Tangerine peel (Powder); 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper.

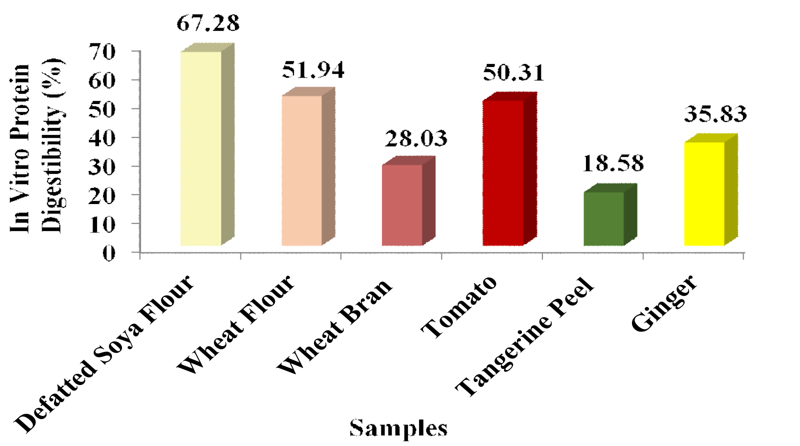
with higher amounts of WF results into higher overall acceptability score of the baked products (Udofia *et al.,* 2013; Ghoshal and Kaushik, 2020; Ayele *et al.,* 2017). These findings are in agreement with Gogoi *et al.* (2023). Sharma and Devi (2021) concluded that a composite flour mixture of water chestnut and soy incorporated at 30% level to wheat flour showed the highest sensory scores during preparation of cookies.

Chemical characteristics of biscuits

Trypsin inhibitor and amino acid content

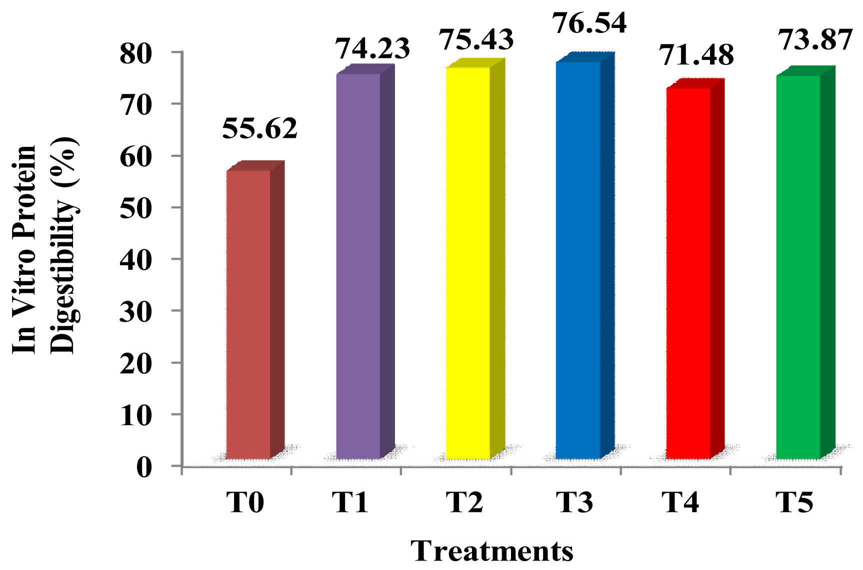
Table 3 represents the trypsin inhibitor content and amino acid composition of raw ingredients and biscuits. Trypsin inhibitor was 4.7 mg/g in defatted soya flour while it was absent in all other ingredients. Toasted defatted soya flour was used during pre sent study and it was reported that trypsin inhibitor residues were pre sent in the flour after toasting (Sessa and Bietz, 1986). In biscuit samples, trypsin inhibitor ranged from 0 to 0.47 mg/g. Trypsin inhibitor was significantly ($p<0.05$) reduced after baking due to its heat labile nature and heating foods at the temperature of 120°C for 15 to 30 minutes reduces almost whole amount of trypsin inhibitor present in legumes which also helps in improving protein digestibility. Higher temperature treatment is required in case of some legumes such as soya bean to reduce this anti nutritional factor (Gopalan *et al.,* 2004). During present investigation, the biscuits containing defatted soya flour were baked at 180°C for 12 minutes. Furthermore, Joshi and Rahal, 2018 stated that processing methods such as germination, fermentation and roasting have the potential to improve nutritional quality of soybean by removing anti-nutritional factors.

Among amino acid, lysine content ranged from 0 to 3.71 g/100 g protein (Table 3). Defatted soya flour and wheat bran contained 3.71 and 3.64 g lysine/100 g protein, respectively. In wheat bran sample, the level of methionine was 0.97 g/100 g protein. Similarly, in WF, defatted soya flour and tomato, methionine content was found at the level of 0.83, 0.71 and 0.42 g/100 g protein, respectively. Observed figures for amino acid content were in accordance with reference values reported by Gopalan *et al.* (2004). Among biscuits, the lowest lysine content was found in T0. In contrast, T_4 and T_5 had higher content of lysine due to addition of DSF and WF. Similar findings were reported by Arshad *et al.* (2007) with regard to increased lysine content (2.32 g/100 g protein) of functional cookies consisting of defatted soya flour at 25% level. Okoye *et al.* (2016) reported the value for lysine content as 4.47 g/100 g protein in wheat



*Values are Mean±SD from triplicate determinations; different superscripts in the same column are significantly different (p<0.05).

Fig 1: *In vitro* protein digestibility of raw ingredients.



*Values are Mean±SD from triplicate determinations; different superscripts in the same column are significantly different (p<0.05).

Where, T_0 = 100% wheat flour ; T_1 = 60% defatted soya flour and 40% wheat flour ; T_2 = 50% defatted soya flour and 50% wheat flour ; T_3 = 40% defatted soya flour and 60% wheat flour ;T_4=20% Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder)7 % Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper; T_5= 20% Defatted soya flour, 60% Wheat flour, 2% Tangerine peel (Powder), 7% Wheat bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper.

Fig 2: *In vitro* protein digestibility of biscuits.

soybean (70:30) composite flour. With regard to methionine content, T_4 contained slightly higher content of methionine as compared toT_5. Olagunju *et al.* (2018) formulated nutritious crackers using WF and pigeon pea in the ratio of 70:30 and observed 2.88 g methionine content per 100 g of protein in the final product. Kamel *et al.* (2020) reported the methionine content as 1.74 g/100 g protein at 20% incorporated defatted soya flour in corn and potato flour during preparation of gluten free crackers.

In vitro protein digestibility

Fig 1 and 2 depict the *in vitro* protein digestibility of raw ingredients and biscuits. Values for *in vitro* protein digestibility of defatted soya flour, WF, wheat bran, tomato, tangerine peel powder and ginger were 67.28, 54.91, 28.03, 50.31, 18.58 and 35.83%, respectively (Fig 1). In pre sent investigation, *in vitro* protein digestibility of control and assorted nutraceutical biscuits showed a significant ($p<0.05$) difference between all samples. The highest (76.54%) *in vitro* protein digestibility was recorded in T_3 while the lowest (55.62%) was recorded in T_0. The protein digestibility was higher (73.87%) in T_5 as compared to that (71.48%) of T_4 in case of assorted nutraceutical biscuit samples (Fig 2). *In vitro* protein digestibility of tempeh from blend of soyabean, green gram and rice flour was ranged between 72.18 and 90.86%, in an investigation undertaken by Lakshmy and Suman (2016). Kumar *et al.* (2018) developed multigrain premix from wheat and soybean blended biscuits and compared those with wheat biscuits. The researchers revealed that multigrain biscuits up to 40% level of pulses supplementation had highest (71.73%) *in vitro* protein digestibility in comparison with control biscuits (38.13%).

CONCLUSION

To retrieve, it can be stated that there is a scope of improvement in level of biscuit nutrition through amalgamation of diverse nutraceutical ingredients. It is hereby recommended that baked snacks/ biscuits can be supplemented using nutraceutical ingredients to foster a healthier dietary lifestyle.

Confiict of interest

We, the authors of this manuscript, declare that there is no conflict of interest for this publication.

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