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(Original Article)

Improving Nutritional Quality of Gluten Free Noodles Using Sand Smelt (*Atherina boyeri*) Fish

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Abstract

The aim of this study was to evaluate gross chemical composition (moisture, protein, fat and ash), mineral content, amino acid profile as well as fatty acid composition of sand smelt (*Atherina boyeri)* fish. Also, to study the effect of incorporation sand smelt mince with gluten free flour to improve nutritional quality of gluten free noodles for coeliac patients. In addition, to study the quality parameters, microbial content as well as sensory evaluation of the prepared gluten free noodles. Sand smelt fish mince contained 80.29 % crude protein, 10.08% crude fat, 9.44% ash and 0.34% carbohydrate (on dry weight basis). Beside 65.22 and 151.82 mg/kg of iron and zinc respectively. Moreover, sand smelt meat contained 39.23mg /100g protein of essential amino acids. Leucine, lysine and valine were the dominant essential amino acids while, glutamic acid was the major non-essential amino acid. Regarding to fatty acids profile, the total unsaturated fatty acid recorded 60.99% of total fatty acids. However, docosahexaenoic acid (C22:6n3) was the dominant fatty acid followed by oleic acid (C18:1) in sand smelt meat. Beside the poly unsaturated fatty acids to saturated fatty acids ratio 1.22in sand smelt fish which was much higher than the recommended value (0.4 - 0.5) by WHO in foods. Incorporation of sand smelt mince during preparation of gluten free noodles increased crude protein, crude fat and ash contents and had no significant effect on over all acceptability of the final product.

Keywords: *Sand smelt fish, Gluten free noodles, cooking quality, Coeliac patients.*

Introduction

Sand smelt (*Atherina boyeri*) is a common species in the Mediterranean Sea. (Altun, 1999). Sand smelt is a rich material in unsaturated fatty acids, protein as well as being a good source of vitamins, and minerals for healthy diet (Bartulovic *et al*., 2004 and Bilgin *et al*., 2011). Adding fish to snacks in various levels is considered as a way of fortification (Nawaz *et al*., 2019). Bissaria (the locale name of sand smelt) is an uneconomical fish and is considered a cheaper species of fish in Egypt (El-Lahamy *et al*., 2019).

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Noodles and pasta-based products are consumed all around the globe, and it traditionally prepared using wheat flour as major ingredients (Nawaz *et al*., 2020). Incorporation of fish meat in snack, especially in noodles, has potential in order to move towards a healthy nutrition (Foschia *et al*., 2015).

Coeliac disease or gluten-related disorders including, wheat allergy and non-celiac gluten sensitivity cause major health problems for people when ingesting small amount of gluten (Conte *et al*.,2019). Because coeliac people have various nutrient deficiencies, there are many challenges in the development of gluten-free products (Saturni *et al*., 2010 and Vici *et al* .,2016).According to statistical findings, approximately 1% of the world population has celiac disease(Malalgoda and Simsek,2017).Since there is no cure for celiac patients, lifelong gluten-free diets are required (Rubio-Tapia *et al*.,2013).Following a gluten-free diet generally causes various nutrient deficiencies. For this reason, developing gluten-free product formulations for pasta that are nutritious and economic are very important Jnawali *et al*., 2016). Thus, this study was aimed to improve the nutritional quality of gluten free noodles by replacing of gluten free flour with 15% sand smelt fish meat.

Materials and Methods

Fresh sand smelt (*Atherina boyeri*) fish was obtained from Abou Qeer fish market, Alexandria, Egypt during 2022. Fish were transported in ice to the laboratory. Sand smelt fish head off, gutted, and washed (cold water). Fish was minced by a kitchen meat mincer with a 3 mm diameter perforated plate. Part of sand smelt mince was directly used for the chemical analysis and the others were packaged in polyethylene bags and kept in deep freezer at-18±1˚C until use for noodles preparation. Samples were thawed overnight at 5±1˚C before use.

Gluten free flour consisted of (potato starch, corn flour and rice flour) was obtained from Awlad Rajab markets in Assiut City, Egypt in 2022.

Spices mixture, salt and sunflower oil were obtained from local markets of Assiut city, Egypt in 2022.

Chemicals

The chemicals and microbial media were obtained from EL Gamhouria for Trading Chemicals and Drugs Co., Assiut city, Egypt.

Methods

Preparation of gluten free noodles

Gluten free noodles were prepared using the formulae (Table1) according to Shikha *et al*., (2020) as control formula as well as 15% sand smelt mince in fortification formula which found to be the most palatable ratios according to the sensory evaluations carried out.

Table 1. Formula of prepared noodles

Ingredients	Sample	
	Control	Fortified formulae
Gluten free flour (g)	100	85
Fish mince(g)	0	15
Water (ml)	40	40
Oil (ml)	5	5
Salt (g)	2	2
Spices (g)	0.5	0.5

All ingredients, as indicated in Table (1), were mixed and noodles samples were prepared according to the methed described by Shikha *et al*. (2020).

Nutrition quality

Chemical composition (moisture, crude protein, crude fat and ash contents) for sand smelt fish, gluten free flour and noodles samples were determined according to official methods (AOAC, 2000), carbohydrates were calculated by difference according to Turhan *et al*. (2005) while caloric value was calculated as multiply the content of protein and carbohydrates by 4 and by 9 for fat content.

Minerals content of sand smelt fish was analyzed. Microwave digestor (Multiwave GO Plus 50 HZ) was used prior to spectrophotometric analysis of the samples by MPAES4210 (Microwave Plasma -Atomic Emission Spectroscopy) (Agilent, Mulgrave, Victoria, Australia). according to Agilent Technologies, Inc. (2021).

Amino acids content of sand smelt fish was determined according to the method described by Pellett and Young (1980).

Tryptophan was determined using spectrophotometric method as described by Sastry and Tummuru (1985).

Fatty acid composition of sand smelt fish

The methyl esters of fatty acids were prepared as mentioned by Rossell *et al*. (1983) and fatty acid composition was determined according to Khalifa (1995).

Physicochemical properties of the prepared gluten free noodles

The method described by Shikha *et al*. (2020) was used for cooking the prepared gluten free noodles.

Water absorption and cooking loss were determined according to the AACC-approved method 66–50.01 AACC (2000).

Microbiological quality

The total plate bacterial counts were determined using the plate counts technique on a nutrient agar medium according to procedures by APHA (1976) and Difco-Manual (1984).

Yeast and Mold counts (YMC) were determined using Bacto yeast malt (Y.M) agar medium according to the methods described by Difco-Manual (1998).

Sensory quality

After cooking noodles were evaluated for color, taste, flavor, texture and overall acceptability by 10 staff members of Agriculture Research Station in Assiut city using 10-point hedonic scale according to Gelman and Benjamin (1989).

Statistical analysis

The data obtained from three replicates were analyzed by ANOVA using The SPSS statistical package program, and differences among the means were compared using the Duncan's Multiple Range test (SPSS, 2020). A significance level of 0.05 was chosen.

Results and Discussion

The Proximate composition of sand smelt mince and the used gluten free flour is given in Table (2). Sand smelt fish meat contained 79.66% moisture, 80.14% crude protein, 10.08% crude fat, 9.44% ash and 0.34% carbohydrates. While the gluten free flour recorded 10.60, 7.46,0.72, 0.51 and 91.31% for moisture, crude protein, crude fat, ash and carbohydrates, respectively. The obtained results for chemical composition of sand smelt meat are in agreement with those reported by Kalogeropoulos *et al*. (2004) and Bilgin *et al*. (2011). Generally, there were significant differences ($p<0.05$) between the main used ingredients (sand smelt mince and gluten free flour) in their chemical composition (Table 2)

Table 2. Proximate chemical composition of the used ingredients (% on dry weight, except moisture)

Parameters	Sand smelt mince	Gluten free flour
Moisture	$79.66^{a}\pm0.03$	$10.60^{b}\pm0.02$
Crude protein	$80.14^{a}\pm0.05$	$7.46^{b}\pm0.01$
Crude fat	$10.08^{a}\pm0.01$	$0.72^{b}\pm0.02$
Ash	$9.44^{a}\pm0.02$	$0.51^{b}\pm0.02$
Carbohydrates	$0.34^{b}\pm0.03$	$91.31^{a}\pm0.05$

Different letters in the same raw means significant differences ($p<0.05$)

Data in Table (3) revealed that there were ten minerals in raw sand smelt meat. The high calcium content (67285.27mg/kg) might be due to including the bone with the minced meat. Potassium was found to be 2766.09 mg/kg in sand smelt mince. Potassium helps in normal functioning of nerves, muscles and heart, sugar metabolism, acid-base balance, and oxygen of brain (Rasul *et al*., (2021). Sand smelt meat contained 28545.34 mg/kg Phosphorous. Which helps in many physiological processes according to Nair and Mathew (2001).

Considerable amount of zinc is present in sand smelt fish (151.82mg/ kg), generally, zinc plays an important role in the immune system and growth and development (Jónsson *et al*., 2007). Sand smelt mince contained 65.22 mg/kg iron.

Iron is an important constituent of myoglobin and the cytochromes (Chandra, 1990). The same data (Table, 3) revealed that, sand smelt fish contained Mg 245.50 mg/kg and Mn 38.69 mg/kg. Magnesium is an active component of several enzyme systems; besides, manganese is a cofactor of hydrolase, decarboxylase, and transferase enzymes. (Murray *et al*., 2000). The copper content was 4.07 mg/kg. Copper is important for many enzymes and it plays a role in iron absorption (Chandra, 1990). Small amount of chromium (0.45 mg/kg) was presented in sand smelt fish meat. Moreover, chromium deficiencies may exist, particularly in children suffering from protein-calorie malnutrition (Mertz, 1974). According to mineral analysis of sand smelt meat, incorporation of such material in Coeliac patients' diets will be very useful.

Table 3. Minerals content of the sand smelt fish (mg/Kg)

Minerals	Sand smelt fish(mg/kg)
Ca	67285.27
K	2766.09
Na	6199.66
P	28545.34
Fe	65.22
Mg	245.50
Zn	151.82
Mn	38.69
Cr	0.45
Cu	4.07

Amino acid composition of sand smelt mince (g/100g protein) is presented in Table (4). As it is expected all essential amino acids are present. However, the dominant essential amino acids were leucine, lysine, valine and threonine. Junianto *et al*. (2022) reported that leucine is beneficial for maintaining nitrogen balance in adults and growth of children. Tryptophan recorded 1.02 g/100g protein and the total essential amino acids recorded 39.23g/100g protein in sand smelt mince. Moreover, total essential amino acids in sand smelt mince were higher than in the FAO/WHO standard (33.9 g/100 g) protein. On the other hand, the total nonessential amino acids were 60.31 g/100 g protein. The dominant non-essential amino acid was glutamic acid which found to be 17.02g/100g protein. Glutamic acid turns in the body into glutamate which helps nerve cells in the brain send and receive information from other cells. However, EAA/ N EA ratio was 0.65 as indicated in Table (4). From such results for amino acid composition of sand smelt, its incorporation into gluten free noodles will improve its amino acids profile.

Table 4. Amino acids composition of sand smelt fish (g/100g protein)

Amino acid	Sand smelt fish mince g/100g protein
Aspartic	10.01
Serine	4.03
Glutamic	17.02
Glycine	7.13
Alanine	7.24
Histidine	2.28
Arginine	5.33
Proline	3.65
Cystine	0.56
Tyrosine	3.06
Total non-essential amino acids	60.31
Lysine	7.93
Leucine	8.39
Isoleucine	4.89
Methionine	1.38
Phenylalanine	4.72
Threonine	5.16
Tryptophan	1.02
Valine	5.74
Total essential amino acids	39.23
Total amino acid	99.54
EAA/ N EA ratio	0.65

EAA = Essential amino acids, NEAA= Non-essential amino acids

Fatty acid profile of sand smelt mince.

The fatty acids profile of sand smelt mince is presented in Table (5). The total saturated fatty acids were 26.84%. The dominant saturated fatty acid was palmitic acid (C16:0) which recorded 16.22% of total fatty acids (TFAs) while the total unsaturated fatty acids (TUSFA) content was 60.99% of TFAs. However, docosahexaenoic acid was the dominant unsaturated fatty acid 19.88% of TFAs) followed by oleic fatty acid (13.23% of TFAs). According to Horrocks and Yeo (1999), docosahexaenoic acids are essential for the growth and functional development of the brain in infants as well as required for the maintenance of normal brain function in adults. Oleic acid was reported as an anti-inflammatory fatty acid playing a role in the activation of the different pathways of immune competent cells (Carrillo *et al*.,2012). As indicated in Table (5), linoleic (18:2) comes in the third order of unsaturated fatty acids content of sand smelt mince recorded at 3.09 % followed by linolenic acid (18:3) with 4.39% of TFAs. However, unsaturated fatty acids are precursors of a group of hormones that play an important role in muscle contractions and the proper healing of inflammatory processes (Coultae, 1989). Generally, fat of sand smelt mince contains a considerable amount of omega-3 and omega-6 fatty acids, which are very important in the development of the child's brain and lower blood pressure to avoid stroke (Junianto *et al*., 2022). As recommended by WHO, 2003 the minimum

PUFA/SFA ratio could be 0.4 – 0.5 in foods, however, it was 1.22 in sand smelt mince which is high quality in this concern. The obtained results for fatty acid composition are in the same line with that reported by Yavuzer (2020) and Balikcl (2021).

Table 5. Fatty acids composition of smelt fish (as % of total fatty acids)

Fatty acids	Carbon Chain	Sand smelt fish mince % of total fatty acids
Lauric acid	C12:0	0.06
Tridecylic acid	C13:0	0.22
Myristic acid	C14:0	3.42
Pentadecanoic	C15:0	0.68
Palmitic acid	C16:0	16.22
Margarinic acid	C17:0	0.97
Stearic acid	C18:0	5.04
Arachidic acid	C20:0	0.14
Docosanoic acid	C22:0	0.05
Lignoceric acid	C:24	0.04
Myristioleic acid	C14:1	0.86
14, Pentadecanoic acid	C15:1	0.27
Palmitoleic acid	C16:1	11.68
Heptadecenoic acid	C17:1	1.59
Oleic acid	C18:1	13.23
Cis-11- Eicosenoic acid	C20:1	0.49
Linoleic acid	C18:2	3.09
	C18:2T	2.98
γ- Linolenic acid	C18:3n6	0.55
Linolenic acid	C18:3n3	4.39
Eicosadienoic acid	C20:2	0.51
Eicosatienoic acid	C20:3n6	1.08
Eicosadienoic acid	C22:2	0.39
Docosahexaenoic acid	C22:6n3	19.88
TSFA		26.84
TUSFA		60.99
MUSFA		28.12
PUSFA		32.87
PUFA/SFA ratio		1.22

TSFA= total saturated fatty acids, TUSFA= total unsaturated fatty acids, MUSFA= mono unsaturated fatty acids, PUSFA= Poly unsaturated fatty acids, PUFA/SFA= Poly unsaturated fatty acids : saturated fatty acids,

Effect of incorporation of fish on the prepared gluten free noodles

Effect of incorporation of fish mince on chemical composition and caloric value of the prepared gluten free noodles are present in Table (6). Compared with control, incorporation of sand smelt led to significant increment of moisture, protein, fat and ash in the prepared noodles. This increment might be due to the high content of these materials in fish meat than gluten free flour (Table, 2). On the other hand, there was significant decrement in carbohydrates and caloric value in the fortified gluten free noodles than control sample due to the low carbohydrate content in sand smelt fish (0.34% on dry weight). The obtained results are in the same line with More *et al*. (2019), who prepared noodles with quail minced meat and Shikha *et al.* (2020) with silver carp fish.

Table 6. Effect of incorporation of fish on chemical composition and caloric value of the prepared gluten free noodles (% on dry weight, except moisture)

Parameters	Noodle (Control)	Noodle (Fortified formula)
Moisture	$6.31^{b}\pm 0.01$	$9.94^{a}\pm 0.02$
Crude protein	$11.95^{b}\pm0.03$	$19.02^{a}\pm001$
Crude fat	$3.59^{b}\pm0.02$	$3.89^{a}\pm0.01$
Ash	$2.27^{b}\pm0.02$	$2.95^{a}\pm0.05$
Carbohydrates	$82.19^{a}\pm0.01$	$74.14^{b}\pm0.01$
Energy (K. Cal/100g)	$408.84^{a}\pm 0.05$	$407.65^{b}\pm0.02$

Different letters in the same raw means significant differences (p<0.05)

Cooking parameters of prepared gluten free noodles:

The different noodle samples were analyzed for the cooking parameters and the results are presented in Table (7). Cooking time was decreased with replacement of gluten free flour by sand smelt fish. Similar trend was reported by Shikha *et al*. (2020) who used silver carp fish meat for prepared noodles. Regarding water absorption and cooking loss (%), incorporating sand smelt meat led to significant decrement. Also, Nawaz *et al*. (2020) found that the loss rate % was decreased with increment of fish meat during preparation of noodles.

Table 7. Cooking quality of the prepared gluten free noodles

Sample	Cooking time needed (min)	Weight of noodles before cooking(g)	Weight of cooked noodles (g)	Water absorption (%)	Cooking loss (%)
Control	5	100	$213^{a}\pm1.53$	$113^{a}\pm1.15$	$5.13^{a}\pm0.02$
Fortified formula	4	100	$195^{b}\pm0.58$	$95^{b}\pm1.53$	$3.75^{b}\pm0.02$

Different liters in the same columns mean significant differences (p<0.05)

As shown in Table (8) total bacterial count (cfu/g×10^4) was significantly increased as incorporation of sand smelt meat during preparation of gluten free flour noodles, and that might be due to the higher bacterial load in fish meat compared to gluten free flour. On the other hand, incorporation of sand smelt fish during noodle preparation had no significant effect on its content of yeast and molds. The obtained results are in the same line with the results of More *et al*., (2019) who found an increment of total bacterial count in prepared noodles with increment of quail meat incorporation. Besides, the maximum acceptable limit is (6.0 CFU/g) for the TPC in the fresh noodles and it may be considered as a reference point between spoiled and unspoiled noodles (Ghaffar *et al*., 2009).

Table 8. Microbial quality of prepared gluten free noodles (log cfu/g)

Noodle samples	Total bacterial count (TPC)	Yeasts and Molds count	
		Molds	Yeasts
Control	1.16^{b}±0.01	1.00^{a}±0.001	1.00^{a}±0.02
Fortified formula	1.36^{a}±0.01	1.00^{a}±0.01	1.00^{a}±0.01

Different letters in the same columns mean significant differences ($p<0.05$)

Sensory evaluation of the prepared gluten free noodles as incorporated with sand smelt meat is presented in Table (9). However, taste, odor and color were decreased but significant differences were found in texture as well as overall acceptability. On the other hand, the taste, odor and color were still accepted by panelists who gave those parameters more than 9 out of 10 on hedonic scale.

Table 9. Sensory evaluation of the prepared gluten free noodles

Sample	Taste	Odor	Texture	Color	Overall acceptability
Control	9.41^{a}±0.21	9.78^{a}±0.23	9.50^{a}±0.09	10^{a}±0.01	9.50^{a}±0.20
Fortified formula	9.32^{b}±0.09	9.20^{b}±0.13	9.41^{ab}±0.18	9.48^{b}±0.13	9.39^{ab}±0.08

Different letters in the same columns means significantl differences ($p<0.05$)

Conclusions

Sand smelt fish contain considerable amount of crude protein with good amino acid profile and its lipid contained high ratio of omega-3 and omega-6 fatty acids as well as many important minerals. Incorporation of sand smelt mince during preparation of gluten free noodles increase crude protein, crude fat andash contents in the final product and improves its nutritional quality for coeliac diseases patients.

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تحسين الجودة الغذائية للمكرونة خالية الجلوتين باستخدام اسماك البيساريا

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الملخص

هدفت الدراســـة الى تقييم التركيب الكيميائى العام (الرطوبة، البروتين، الدهن والرماد)، والمحتوى المعدنى وتركيب الأحماض الامينية بالاضـــافة الى تركيب الأحماض الدهنية في ســمك البيســاريا (*Atherina boyeri*) أيضــا دراســة تأثير دمج مفروم ســمك البيســاريا مع الدقيق خالى الجلوتين لتحسين الجودة الغذائية للمكرونة خالية الجلوتين لمرضى السلياك. الى جانب ذلك دراسة خصـــائص الجودة والمحتوى الميكروبى والتقييم الحســـي لهذه المكرونة. احتوى مفروم ســـمك البيســاريا على 80.13 بروتين ،10.08% دهن, 9.44% رماد،0.34% كربوهيدرات (على اساس الوزن الجاف) اضـــافة الى ذلك احتوى 65.22 و151.82مجم/ كجم من الحديد والزنك على التوالى. بالأضـــافة الى ذلك فقد كان محتوى ســمك البيســـاريا من الأحماض الأمينية الأســـاســية 39.23مجم / 100 جرام بروتين. وكانت احماض الليوســـين والليســـن والفالين هى الأحماض الأمينية الضـــرورية الســائدة، بينما كان حمض الجلوتاميك هو الرئيســـى الأحماض الأمينية غير الضـــرورية. وفيما يتعلق بتركيب الاحماض الدهنية، فقد ســجلت الأحماض الدهنيه غير المشــبعة 60.99% من الأحماض الدهنية الكلية. كان الحمض الدهنى دوكوزاهكســـانيونيك (C22:6n3) هو الســائد بين الأحماض الدهنية غير المشــبعة يليه حمض الأوليك (C18:1) فى سـمك البيسـاريا. الى جانب ذلك كانت نسبة الأحماض الدهنية عديدة عدم التشبع الى الأحماض الدهنية المشبعة فى هذا الســـمك هى1.22 والتى كانت أعلى كثيرا من القيمة الموصـــى بها فى الأطعمة بواســـطة منظمة الصـــحة العالمية (0.4- 0.5). واخيرا فقد أدى دمج مفروم ســمك البيســـاريا خلال اعداد المكرونة خالية الجلوتين الى زيادة محتوى كل من البروتين والدهن والرماد فى المنتج النهائى دون حدوث تأثير معنوى على درجة التقبل العام له.