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Enrichment of Food with Freeze-Dried Sea Fennel (Crithmum maritimum L.)

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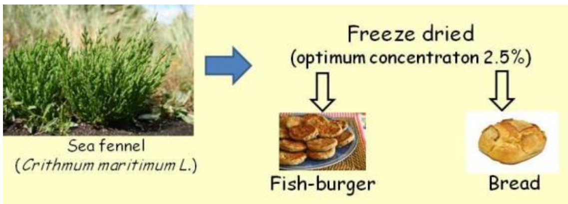
Graphical Abstract



Abstract

The halophytic aromatic plant, sea fennel (Crithmum maritimum L.) (named as SF), has moderate content of Na+. Therefore, in this study SF freeze-dried was used to enrich bread (Br-SF) and fish burger (Fb-SF), without using common salt in the formulation. Specifically, the effects of SF addition on sensory quality, cations (Na+, Ca2+, Mg2+ and K+) content, and the bio accessibility were evaluated. The SF was added to the products in different concentrations from 2.5% (w/w) until the sensory quality reached the threshold value above all in the taste. The best concentration of SF identified was 2.5% (w/w) in both products. Results obtained highlighted that its addition to bread allowed reducing the Na+ content by about 79% than unfortified sample. The fish fortification with SF allowed increasing the Ca2+ content, whereas the Ca2+ bio accessibility from fortified bread underline the possibility to increase the Ca2+ adsorbed in intestinal tract during the digestion process.

Keywords: *Crithmum maritimum L.; Halophytic aromatic plant; Bread; fish burger; Cations bio accessibility; Fortified food.*

Introduction

Nowadays, the new life style has considerable impact on human health, the people of worldwide are always more concerned about the quality of food and beverages that they consume. Therefore, the change in consumer outlook has encouraged the food industry to apply modern manufacturing technology to enhance food quality such as food fortification [1,2]. As defined by Allen et al. (2006) [3], this practice allows to improve the nutritional quality of foods through the addition of essential micronutrients, vitamins and minerals, so makes available beverages and common foods, like soups, yogurt, sauces, jams, cheese or bread that can provide a public health benefit with minimal risk to health.

The fortification vehicle must be either a staple food that is widely consumed throughout the year by a large portion of the population. Besides the selection of an appropriate food vehicle and its end use for a successful application of fortification technology it must also be considered the nature of the food product, its production process and the type of fortifying compound [4]. A food can only be fortified to a level that does not change its sensory and physical properties. This level should be experimentally determined and tested by experts in sensory analyses to determine the amount of each nutrient technically compatible with a specific food matrix. Various technological strategies have been developed to solve some of these problems (microencapsulation of protective coverings; use of stabilizers and emulsifiers to maintain the mineral in solution), but some difficulties remain and other types of fortification or integration should be considered. To overcome problems of flavor, texture and color deterioration due to bio-ingredients addition, some studies have engineered new fortification strategies that involve the use of enriched herbal extracts and plants. Plants produce a multitude of chemical compounds; most of them are biologically active in human beings [5], while herbs are naturally rich in minerals, vitamins, flavoring agents and natural antioxidants [6-9]. Hence, their use for fortification may represent a new trend to improve the nutritional quality of foods products. Sea fennel (*Crithmum maritimum* L.) is a halophytic aromatic plant that growth wild along the Mediterranean coast. Several uses of this plant are known for culinary purpose and its leaves used as tonic, carminative, diuretic and vermifuge effects [10]. This wild edible plant is deserving a special consideration in the field of food fortification technology, since it is possible to use the sea fennel as a new spice-colorant in culinary preparations, in order to improve the nutritional quality [11,12]. Sea fennel plant has various nutritional interests due to its high contents in different bioactive compounds, such as: flavonoids, carotenoids, vitamin C and others substances with beneficial properties [13]. Starting from these considerations the aim of this study was to develop new fortified food with SF (bread and fish-based products). To the aim, both sensory and nutritional quality were assessed. In addition, K^+, Mg^{2+} and Ca^{2+} bio accessibility by *in vitro* gastro-intestinal digestion was measured.

Materials and Methods

Raw materials

To obtain freeze-dried Sea Fennel (SF), the aerial part of plant was randomly collected from many plants along the shoreline in Mola di Bari (Bari, Italy). Then, the plant material was immediately transferred to the postharvest laboratory of the Institute of Sciences of Food Production-National Research Council of Italy-and was subsequently cleaned and separated into "edible" and "refuse portion" categories. The latter are generally consisting in the older leaves and fibrous stems that are removed during normal food preparation. The fresh material was firstly frozen at -23°C and then it was freeze-dried for 72 hours with a condenser temperature of about -52°C. For freeze-drying (FD), a laboratory freeze dryer (LABCONCO Free Zone® Freeze Dry System, model 7754030, Kansas City, USA) equipped with a stoppering tray (LABCONCO Free Zone® Stoppering Tray Dryer, model 7948030, Kansas City, USA) was used. The dehydrated material was grinded (1 min; 0.78 G) in a blender (Sterilmixer lab, International PBI, Milan, Italy), to obtain the powder. So, the powdered sea fennel was packed in glass jars closed with an air-tight cap and stored at-20°C without light, before being used to prepare bread and fish burgers. Wheat flour for bread production was purchased from Agostini mill (Molini Agostini s.r.l., Ascoli Piceno, Italy), compressed fresh yeast, sugar and salt were bought from local market. Frozen swordfish (Xiphias gladius) (around 10 kg) was kindly provided by a local fishing company (Caggianelli, Bisceglie, Italy), delivered to the university laboratory and kept in a freezer at-18°C. Before use, fish has been thawed at 5°C for 24 hours in a refrigerated chamber and sliced.

Samples preparation

Bread-making process (dough mixing, processing and baking) was performed on laboratory-scale equipment and a straight dough method was used. The dough based on wheat flour (100%) used as reference sample (named as Br-CTRL), was prepared as follows: 1500 g of wheat flour, 900 g of water, 45 g of compressed fresh yeast, 15 g of sugar and 24 g of salt. Firstly, fresh compressed yeast was dissolved in a part of water (300 g) and was placed in the incubator (Thermogel, Varese, Italy) at 30°C and 85% relative humidity (RH) for 30 min. After the fermentation, the paste was slowly mixed with all other ingredients. The dough was then mixed using a mixer (Conti Impastatrici, Verona, Italy) at low speed (110 x g) for 5 min, followed by 10 min of mixing at high speed (200 x g). After complete mixing, dough was placed in the incubator at constant temperature (30°C) and at 85% RH for 60 min. After the first proofing, dough portions of 800 g were manually rounded and placed in the incubator for the last 30 min under the same incubation conditions. Finally, after the second proofing, dough samples were baked in a pre-heated electric oven (Europa Forni, Vicenza, Italy) at 230°C for 15 min, followed by 35 min at 200 °C. Baking process was performed in triplicate. Samples were left to cool at room temperature for about 2 h and were subsequently submitted to sensory and chemical analyses. The fortified experimental bread samples were prepared using the same procedure as for the Br-CTRL sample with the following changes: the salt elimination from the formulation and addition of freeze-dried powder SF at different concentrations of 2.5%, 5%, 10% and 15% (samples were named as Br-SF2.5; Br-SF5; Br-SF10; Br-SF15, respectively). All percentages were calculated on the total weight flour.

As regard fish burgers, the samples were elaborated in a laboratory pilot plant. Swordfish fillet slices were minced by an industrial food processor (Everest, Rimini, Italy) before being added to the mixture. Ingredients were added as follows (g/kg of raw material): minced fish (765), extra-virgin olive oil (100), NaCl (5), parsley (5), rosemary (5), curry powder (5), potato starch (50) and potato flakes (65). All the ingredients were homogenized in a bowl mixer (Multichef, Ariete, Firenze, Italy) equipped with a spiral hook for 5 min. The obtained fish dough was loaded with different concentrations of SF (2.5%, 5% and 7.5%) (w/w), homogenized for a few minutes and then shaped by hand to obtain a fish burger (40 g, 30-40 mm diameter). The investigated samples were labeled as Fb-SF2.5; Fb-SF5 and Fb-SF7.5, respectively. As control sample, fish burger without SF was also realized (Fb-CTRL). To improve clarity about the nomenclature, all the investigated samples were labelled as listed in (Table 1).

Sample	Abbreviation
Freeze-dried sea fennel powder	SF
Control bread	Br-CTRL
Bread fortified with 2.5% of freeze-dried SF	Br-SF2.5
Bread fortified with 5% of freeze-dried SF	Br-SF5
Bread fortified with 10% of freeze-dried SF	Br-SF10
Bread fortified with 15% of freeze-dried SF	Br-SF15
Control swordfish burger	Fb-CTRL
Fish burger fortified with 2.5% of freeze-dried SF	Fb-SF2.5
Fish burger fortified with 5% of freeze-dried SF	Fb-SF5
Fish burger fortified with 7.5% of freeze-dried SF	Fb-SF7.5

Table 1: Experimental samples.

Extraction and analysis of nitrate and soluble sugar assay in sea fennel

Ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was performed as reported by D'Imperio et al. (2016) [14] for NO_3^- determination. Briefly, the NO_3^- were extracted from 0.5 g of freeze-dried powder SF samples with 3.5 mM Na_2CO_3 and 1 mM $NaHCO_3$ for 30 minute. After extraction, the samples were diluted and filtered by using 0.45 μm (RC) followed by Dionex OnGuard IIP (ThermoScientific) in order to remove organic compounds (phenolic fraction of humic acids, tannic acids, lignins, anthocyanins, and azodyes from sample matrices). The resulting solutions were analysed by ion chromatography with a conductivity detector by using an IonPac AG14 precolumn and an IonPac AS14 separation column (Dionex Corporation, Sunnyvale, CA). In order to determine glucose, fructose and sucrose contents, samples were prepared with protocols used by Renna et al. (2014) [15] and analysed by ion chromatography (Dionex DX-500; Dionex Corp., Sunnyvale, CA) with a pulsed amperometric detector. Peak separation was performed using a Dionex CarboPac PA1 and isocratic elution with 50 mmol L^{-1} NaOH.

Extraction and quantification of inorganic iodine in sea fennel

The inorganic I^- determination was made using the protocol of Perring, Basic-Dvorzak, & Andrey (2001) [16]. Briefly, a representative quantity of freeze-dried SF powder was taken and the I^- was extracted with hot water (60 °C) and stirred for 30 min. Then, the sample was allowed to cool down to room temperature. The product solution was well mixed and filtered through an ash less filter paper followed by a 0.2 μm membrane filter. As regard the quantification of inorganic I^-, a representative quantity of extract was used for colorimetric reaction as reported by Perring, Basic-Dvorzak and Andrey (2001) [16]. The absorbance of sample was determined at 454 nm. The standards for inorganic I^- analysis were made from a 100 μg L^{-1} I^- stock solution, standard concentrations ranged from 12 to 0 μg L^{-1}. The quantification of inorganic I^- in SF sample was determined by interpolation with a calibration curve, previously made with an R^2 = 0.9899.

Extraction and analysis of total polyphenols in sea fennel

The Total Polyphenols (TP) content in freeze-dried SF powder was determined by using the Folin Ciocalteu method, previous extraction by using the methods report by Luthria et al. (2006) [17] with some modifications. Briefly, approximately 200 mg of freeze-dried SF powder was mixed with 10 mL of the solvent mixture MeOH: H2O:CH3COOH (79:20:1% v/v/v). The vials were then placed in a sonicator bath at ambient temperature for 30 min, followed by 1 hour in magnetic stirred. The mixture was centrifuged at 10.000 x g, 4°C for 10 min and the supernant was transferred into a volumetric tube. The residue was re-suspended in 10mL of MeOH: H2O:CH3COOH (79:20:1% v/v/v), gently mixed manually and sonicated for an additional 30 min followed stirring (1 h) and centrifugation (10.000 x g, 4°C 10 min). The supernant was combined with the initial extract and appropriate aliquots of extracts were filtered and assayed by the Folin Ciocalteu method for TP content. For each sample, triplicate extractions and analyses were carried out. The content of TP was determined using the Folin Ciocalteu assay with gallic acid (R2 = 0.9921) as a calibration standard by using a Perkin- Elmer Lambda 25 spectrophotometer (Boston, MA, USA).

Extraction and analysis of Na^+, K^+, Mg^{2+} and Ca^{2+} in sea fennel and food

An ion exchange chromatography (Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector was performed as reported by D'Imperio et al. (2016) [14]. For the determination of cations contents (Na^+, K^+, Mg^{2+} and Ca^{2+}), 1 g of dried sample (SF, fortified and unfortified products) was burned in a muffle furnace at 550°C (six hours for SF, Br-CTRL, Br- SF2.5 and 24 hours for Fb-CTRL, Fb-SF2.5 cooked and uncooked) and digested with 1 M HCl in boiling water (99.5±0.5°C) for 30 min. The resulting solution was filtered, diluted and analysed by ion chromatography (Dionex DX120, Dionex Corporation) with a conductivity detector using an IonPac CG12A guard column and an IonPac CS12A analytical column (Dionex Corporation) at 35 °C, flow 1 m min^{-1}.

Sensory analysis

Sensory evaluation was carried out on both bread and fish-burger samples by 10 trained tasters (researchers of the Department of Agricultural Sciences, Food and Environment of the University of Foggia). Our panellists had at least several years of experience in food evaluation prior to this study. The samples (bread or fish burgers) were placed on white plates, identified with random three-digit numbers and evaluated in different sessions. The bread samples were sliced with an electric slicing knife (thickness of 15 mm) (Atlantic; Calenzano, Firenze, Italy) without removing the crust. The evaluators were asked to give a judge on color, odor, appearance, crust and crumb firmness, large bubbles and overall quality. For the sensory analysis of fish-burgers, the judges were asked to evaluate odor, color, appearance, texture and overall quality of uncooked samples. In addition, the fish taste was evaluated after cooking (electric oven, 20 min x 240°C).

A 9-point scale was used to quantify each attribute for both types of products, where a score of 9 corresponded to "very good quality," scores of 7-8 to "good quality" and a score of 6 to "sufficient quality." The value equal to 5 was taken as the lowest limit of acceptability, while a score of 1-4 corresponded to "unacceptable quality."

Assessment of cations bio accessibility

The assessment of cations bio accessibility (percentage of ions, K^+, Mg^{2+} and Ca^{2+} released from food samples during *in vitro* gastro-intestinal digestion) was carried out as described by Ferruzzi, et al. (2001) [18] with some modifications about mouth phase. Briefly, 0.20 g of food samples (dry) were homogenized with 4.5 mL of 0.9% NaCl and α-amylase 3000 units. Samples were flushed with N_2 and incubated at 37°C, then mixed (1 × g at 37 °C) for 10 min in a Rotator Type L2 (Labinco BV, Breda, Netherlands), at the initial pH 7.0. The gastric phase started with the addition of 0.9 mL of pepsin solution (40 mg mL^{-1}) in 0.1 N HCl and adjustment of pH to 2.5 (± 0.1) with 1 N HCl. Samples were flushed with N_2 and incubated at 37°C, then mixed (1 × g at 37°C) for 1 hour in a Rotator Type L2. After the gastric digestion, the small intestinal phase was started by adjusting the gastric digesta pH to 5.3±0.1 with a combination of 100 mM NaHCO3 and 1 N NaOH followed by the addition of small intestinal enzyme solution (2.7 mL of porcine lipase [2 mg mL-1], pancreatin [4 mg mL-1] and bile [24 mg mL-1] in 100 mM NaHCO3). The pH of the final sample was adjusted to 6.5±0.1 with 1 N NaOH; volume was standardized to 15 mL with 0.9% NaCl, and samples were blanketed with N2, incubated at 37°C and mixed for two hours by Rotator Type L2 (1 × g at 37°C).

After the in vitro digestion, the samples were centrifuged at 10.000 x g for 1 hour at 4°C to separate the aqueous intestinal digesta (AQ) from the residual solid. Aliquots of undigested AQ were collected, filtered using a 0.2 μm PTFE filter and successively analysed following the method used for vegetables materials. Blank correction was performed and subtracted in all analyses, in order to reset the contribution of blanks as suggest by Montesano et al. (2016) [19]. The protocol applied **(Ferruzzi et al. 2001)** [18] does not allow evaluating the Na+ bio accessibility. Blank correction was not performed for this cation, because the Na+ released from the food matrix, during in vitro digestion, was very low respect to Na+ present in blank digested sample, probably related to reagents used in this protocol, according to other Authors (Hamilton et al. 2015) [20]. The K+, Mg2+ and Ca2+ content in digested fluids were determined by using the same protocol for the quantification ions in food samples (SF, fortified and unfortified products). The bio accessibility was calculated as follows: (concentration of cations in the intestinal digesta/concentration of cations in food sample) × 100. Statistical analysis Experimental data were compared by one-way variance analysis (ANOVA). A Duncan's multiple range test, with the option of homogeneous groups ($P < 0.05$), was used to determine significance among differences. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this purpose.

Results and Discussion

Nutritional evaluation of freeze-dried sea fennel

The Dry Matter (DM) of SF used in this study was of about 15 g 100 g^{-1}, in agreement with Renna et al. (2017) [12], who reported the same content of dry matter in freeze-dried SF powder. The total sugar content was of 66.1g kg^{-1} DM, with a higher sucrose content respect to glucose and fructose content (Table 2). To our best knowledge there are no data in the literature regarding the sugar content in SF. At the same time, considering a total sugar content of 0.99 g 100 g^{-1} Fresh Weight (FW), it is interesting to highlight the lower content of sugars in SF respect other vegetables of the *Apiaceae* family, such as fennel (*Foeniculum vulgare* Mill.) (3.93 g sugars 100 g^{-1} FW) [21]. The NO_3^- content was of about 98 mg kg^{-1} DM (Table 2). NO_3^- in plants food is an essential parameter for marketable quality of products, since the presence of NO_3^- in plant foods is a serious threat to man's health [22]. The NO_3^- content found in SF was very low respect to standard limit imposed by Commission Regulation (EU) No 1258/2011 for some leafy vegetables and also lower respect to limit imposed by EU for baby foods (200 mg kg^{-1} FM). The low NO_3^- level could be related to the low quantity of nitrogen present in rock soil, the natural habitat for SF plants. The I^- content was of about 4 mg kg^{-1} DM (Table 2), low if consider that this plant generally grows near to sea, however, the results was in line with Shacklette and Cuthbert (1967) [23] relating to others land species. These authors suggest a plants ranking classification in relation to I^- content. Based on this classification the SF could be considered as species with low iodine content. The TP content was of about 28 g kg^{-1}

DM (**Table 2**), in agreement with other authors [24,25]. The level of these beneficial compounds was higher respect to others species, such as spinach and broccoli [26]. In human nutrition, the consumption of plants rich in polyphenols has been associated with providing health benefits by reducing the risk of specific cancers, cardiovascular diseases, and chronic eye diseases. As regards the elements composition of SF, Na^+ was the cation more abundant (about 43 g kg^{-1} DM - Table 2). This result was related to the environmental growth site condition, since the SF usually growth near the sea water.

Glucose	Fructose	Sucrose	NO_3^-	Iodine	Total Polyphenols	Na^+	K^+	Mg^{2+}	Ca^{2+}
	g kg^{-1}			mg kg^{-1}			g kg^{-1}		
8.40±0.62	3.76±0.30	53.92±1.28	98.16±12.81	3.97±0.16	28.28 ±0.85	42.86±2.30	10.09±0.27	3.26±0.14	18.29±0.77

Table 2: Chemical traits of dry matter freeze-dried SF powder.

The contents of K^+ (10 g kg^{-1} DM - Table 2) and Mg^{2+} (about 3 g kg^{-1} DM - Table 2) was in line with the values reported by Renna et al. (2015) [27] for others wild edible plants. On the other hand, Ca^{2+} content (about 18 g kg^{-1} DM-Table 2) in SF was higher respect to values reported by Guil et al. (1996) [28]. It is interesting to highlight that the Ca^{2+} content in SF was higher also in comparison to the *Salicornia europea* L., another typical wild edible plant of the marine coastal habitat (Guil et al. 1998) [29]. Considering all data regarding the nutrient composition, the results of the present study suggest that SF can be considered an interesting ingredient for food industry, since it is a good source of healthy compounds such as polyphenols, Ca^{2+} and I^-, without a high level of NO_3^-, one of the typical anti-nutritional components of leafy vegetables.

Sensory properties of bread and fish-burgers

Fortified food were produced by adding SF powder to bread and fish formulations. As it is well known, during food fortification the ingredients added to improve the nutritional content can compromise sensory acceptability [30-32]. For this reason, sensory evaluations on both bread and fish burger samples were carried out to define the better amount of SF to be added. Results on the sensory properties of the investigated samples are reported in **(**Table 3 and 4). As regard bread, from **(Table 3)** it is possible to infer that fortified samples became unacceptable when more than 10% of SF was added to the formulation. Increasing the SF concentration to 15%, the attribute more compromised is the taste even though also odor, color and texture were very different from the control samples.

Sample	Color	Appearance	Odor	Taste	Crust firmness	Crumb firmness	Large bubbles	Overall Quality
Br-CTRL	$8.05±0.16^a$	$8.00±0.24^a$	$7.90±0.21^a$	$7.80±0.26^a$	$8.10±0.21^a$	$8.05±0.16^a$	$7.75±0.26^a$	$7.95±0.16^a$
Br-SF2.5	$7.80±0.26^a$	$7.65±0.34^a$	$7.20±0.26^b$	$6.50±0.24^b$	$7.85±0.34^{a,b}$	$7.75±0.49^a$	$7.70±0.35^a$	$6.70±0.26^b$
Br-SF5	$7.65±0.34^a$	$7.50±0.41^{a,b}$	$6.75±0.35^{b,c}$	$6.20±0.42^b$	$7.60±0.21^{a,b}$	$7.55±0.16^a$	$7.35±0.41^a$	$6.55±0.28^b$
Br-SF10	$7.10±0.21^b$	$7.05±0.28^b$	$6.25±0.26^c$	$5.15±0.24^c$	$7.30±0.26^b$	$6.80±0.35^b$	$6.50±0.33^b$	$5.65±0.41^c$
Br-SF15	$6.25±0.35^c$	$6.15±0.24^c$	$5.40±0.39^d$	$4.30±0.42^d$	$6.55±0.44^c$	$5.20±0.26^c$	$5.55±0.28^c$	$4.60±0.32^d$

$^{a-d}$ Means in the same column followed by different superscript letters differ significantly ($P<0.05$).

Table 3: Sensory characteristics of the investigated bread samples.

These defects could be most probably being ascribed to the high amount of SF that decreased the capacity to absorb available water with consequent undesired modification of the sensory performance. At very concentration of SF (2.5%) the sensory quality was generally very comparable to the control sample and for this reason the sample with the lowest concentration of SF was taken into account for the further analysis. As regard the fish burgers, in (**Table 4a and 4b)** uncooked and cooked samples can be compared.

Sample	Color	Odor	Appearance	Texture	Overall Quality
Fb-CTRL	7.88±0.23^{a}	7.63±0.58^{a}	7.81±0.26^{a}	7.19±0.26^{a}	7.69±0.26^{a}
Fb-SF2.5	7.63±0.35^{a,b}	7.38±0.58^{a}	7.75±0.27^{a}	7.25±0.27^{a}	7.19±0.37^{a}
Fb-SF5	6.56±0.62^{b,c}	7.06±0.82^{a}	6.50±0.65^{b}	6.31±0.26^{b}	5.94±0.78^{b}
FB-SF7.5	6.06±0.98^{c}	6.69±1.10^{a}	6.00±1.04^{b}	5.19±0.70^{c}	5.00±0.85^{b}

$^{a-e}$Means in the same column followed by different superscript letter are significantly different (P<0.05).

Table 4a: Sensory characteristics of the investigated uncooked fish burger samples.

Sample	Color	Odor	Appearance	Texture	Taste	Overall Quality
Fb-CTRL	7.75±0.46^{a}	7.81±0.26^{a}	7.81±0.37^{a}	7.44±0.32^{a}	7.44±0.32^{a}	7.56±0.18^{a}
Fb-SF2.5	7.56±0.42^{a,b}	7.75±0.27^{a}	7.44±0.50^{a,b}	7.25±0.27^{a,b}	7.13±0.58^{a}	7.19±0.37^{a,b}
Fb-SF5	7.00±0.65^{a,b}	7.06±0.78^{a}	7.06±0.62^{a,b}	6.50±0.58^{b}	6.13±0.92^{a,b}	6.19±0.88^{b,c}
FB-SF7.5	6.50±0.71^{b}	6.69±1.13^{a}	6.44±0.68^{b}	5.56±0.62^{c}	5.19±0.80^{b}	5.13 ±0.83^{c}

$^{a-m}$Means in the same column followed by different superscript letter are significantly different (P<0.05).

Table 4b. Sensory characteristics of the investigated cooked fish burger samples.

From the results can be inferred that no relevant differences were recorded between uncooked and cooked fish burgers. In general, it can be possible to infer that acceptability of this fish-based product is greatly compromised when the SF was used at the highest concentration (7.5%). This was probably due to the water holding capacity exerted by the SF powder, which can increase the product hardness and reduce its juiciness, affecting consequently the *overall quality*. As happens for bread, also in this case, the fish product with lowest amount of SF was selected, being the most similar to the control sample.

Dry matter and cation contents in bread and fish burgers

The SF enrichment did not influence the dry matter, in both bread and fish burgers (66.0 g 100g^{-1}, 41.7 g 100g^{-1} and 44.4 g 100g^{-1} on average, in bread sample, fish-burger uncooked, and cooked samples, respectively). The addition of freeze-dried SF power in bread dough allowed to reduce the sodium content by about 79%, thanks to the elimination of salt from the formulation in Br-SF2.5 (**Table 5**), without significant differences regarding the taste (Table 3).

Sample	Na^+	K^+	g kg^{-1} DM	Mg^{2+}	Ca^{2+}
Bread					
Br-CTRL	5.02±0.06^{a}	1.47±0.27^{a}		0.29±0.02^{b}	0.99±0.11^{b}
Br-SF2.5	1.04±0.12^{b}	1.39±0.02^{a}		0.40±0.01^{a}	1.75±0.10^{a}
Fish-Burger uncooked					
Fb-CTRL	5.68±0.02^{b}	9.25±0.09^{a}		0.79±0.06^{b}	0.45±0.10^{b}
Fb-SF2.5	7.37±0.02^{a}	9.25±0.15^{a}		0.92±0.03^{a}	1.98±0.04^{a}
Fish-Burger cooked					
Fb-CTRL	6.43±0.33^{b}	9.75±0.46^{a}		0.86±0.03^{a}	0.45±0.03^{b}
Fb-SF2.5	7.21±0.77^{a}	9.39±0.19^{a}		0.91±0.03^{a}	2.15±0.11^{a}

$^{a-b}$ Means (±SD) in the same column followed by different superscript letters differ significantly (P<0.05).

Table 5: Cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) contents in fortified and unfortified products (bread and fish-burger).

It is important to highlight that although Na^+ is an essential nutrient, its consumption in excess has been linked to several health problems such as hypertension and cardiovascular diseases [33]. Therefore, the World Health Organization (WHO) recommends the ingestion of less than 2000 mg of Na^+ per day. The results of the present study show that 100 g of bread supply about 331 mg of Na^+ for Br-CTRL and only 69 mg for Br-SF2.5. As regard K^+ content, no differences were found between unfortified and fortified bread samples. On the other hand, the introduction of freeze-dried SF in dough allowed increase the content of Mg^{2+} and Ca^{2+} in bread by about 38% and 77%, respectively (Table 5). The mineral composition of bread was correlated with raw material used, however, the Mg^{2+} content in Br-CTRL found in the current study was similar respect to Mg^{2+} level found in wheat bread reported by others authors [34].

As regard the fish burgers, the introduction of freeze-dried SF increased the content of Na^{+}, Mg^{2+} and Ca^{2+}, respectively by 30%, 16% and 340% in uncooked fish burgers, while in cooked ones the increase was 12%, 6% and 378%. Moreover, similarly to what reported for bread products, no differences were found between unfortified and fortified samples as regard K^{+} content (Table 5). It is interesting to highlight that the Ca^{2+} content in fortified fish burger was higher respect to the average values reported for fish products [35]. Calcium is an essential nutrient for human health, because it is a structural component and takes part in a variety of biological processes. Considering the Ca^{2+} dietary reference intake of 1000 mg per day [36], 100 g of fortified fish burger supply 988 mg of Ca^{2+}, providing almost everything daily intake of this element, while for unfortified samples 100 g of fish burger supply only 207 mg of Ca^{2+}, about the 20% of the daily intake. These results underline that the freeze-dried SF powder enrichment can allow the production of new fortified foods with different contents of some elements with specific nutritional requirements.

Cation bio accessibility of bread and fish-burgers

As reported in **(Table 6)**, the Ca^{2+} bio accessibility of the fortified bread was about 4-fold higher than the unfortified bread. At the same time, the Ca^{2+} bio accessibility found in Br-CTRL was similar respect to what reported by Martins et al. (2017) [37]. On the other hand, no significant differences were observed between fortified and unfortified fish burgers. It may be speculated that the absence of an increase of Ca^{2+} bio accessibility in fortified fish burgers could be due to the food matrix composition. In fact, some studies [38-40] report that different foods showed different values of Ca^{2+} bio accessibility most probably due to the presence in the food matrix of some compounds, such as fiber [41]. Independently to the fortification process, the Mg^{+} bio accessibility was higher respect to that found by others authors [37,38,42]. Concerning the K^{+}, enrichment of freeze-dried SF did not influence the bio accessibility in bread samples, while it was observed its reduction in fish burgers. The bio accessibility assessment is the first point to understand the nutritional efficiency of food fortification process; in fact, this study allows underline that the fortification process of bread and fish burger is able to increase the potentially uptake only in Br-SF2.5.

Sample	Ca^{2+}	Mg^{2+}	K^{+}
Bread			
Br-CTRL	10.75 ±2.07^{b}	61.85±6.11	89.45±9.53
Br-SF2.5	40.05 ±8.39^{a}	70.20±20.78	87.36±9.30
Fish Burger cooked			
Fb-CTRL	48.23 ±19.35	89.14±5.65	80.59±1.94^{a}
Fb-SF2.5	39.80 ±2.75	90.42±3.46	71.87±4.82^{b}

$^{a\text{-}b}$Means in the same column followed by different superscript letter are significantly different (P<0.05).

Table 6: Cations (Ca^{2+}, Mg^{2+} and K^{+}) bio accessibility (%) after *in vitro* gastro intestinal digestion process of bread (fortified and unfortified) and fish burger cooked (fortified and unfortified).

Conclusion

The freeze-dried SF used to fortify both bread and fish burger allowed obtaining food products with similar sensory quality respect to unfortified samples. Sensory evaluation highlighted that adding of 2.5% of SF allowed to obtain the most similar overall quality to the control for both bread and fish- burgers. Moreover, the fortification process improved nutritional profiles by increasing Ca^{2+} and 385 Mg^{2+} contents, generally positively correlated with bone health, and decreased Na^{+} content, correlated with hypertension diseases. Finally, the assessment of cations bio accessibility for bread suggests the possibility to use the fortified bakery products as an alternative calcium source in the daily diet of people affected by milk intolerance and/or with other specific nutritional needs.

Therefore, it is possible to conclude that freeze-dried SF can be considered as an interesting ingredient for food applications in point of view to development of new functional foods.

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