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Enhancement of Technological Properties and Therapeutic Potencies of Couscous through Enrichment with Issoufer: Traditional Plant Medicines

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Abstract

Enrichment of food is a strategy to reduce the incidence of deficiencies in micronutrients in a population. Couscous is widely consumed in Algeria, thus the objective of this study was to obtain an enriched couscous with enhanced therapeutic potencies and with good physicochemical, technological, and microbiological characteristics. Three mass ratios of the traditional preparation Issoufer (10, 20, and 30 %) were added to wheat couscous, and then compared with a control couscous made 100 % of semolina. A significant ($p < 0.05$) increase was noticed in the ash, proteins, lipids, carbohydrates, phenolic compounds, flavonoids, and anti-radical activity values, for all the mass ratios of Issoufer powder. In contrast, the moisture and pH-value revealed a significant ($p < 0.05$) decrease in Issoufer powder. The acute toxicity study revealed no lethal effects and behavioural signs of toxicity at the tested doses (100, 250, 500, and 750 mg kg^{-1}) of the extract of Issoufer during the 5 days of observation. The results of antibacterial activity showed diameters of inhibition zones had achieved 29.90±0.60 mm. Based on obtained results, Issoufer powder can be considered a good ingredient to develop functional couscous naturally enriched of secondary metabolites, and can be used in the prevention of several diseases, as well as used in the food industry.

Keywords

Food enrichment, couscous, Issoufer, antioxidant activity, antibacterial activity

1 Introduction

Enrichment is a strategy to correct deficiencies in micronutrients and reduce their incidence. This technology is justified when access to a diversified diet is limited and therefore adequate levels of various micronutrients are not achievable. The goal of micronutrients is to increase the intake of one or more nutrients that are insufficient in the food supply.[1,2]

Couscous is the product of durum wheat semolina (*Triticum durum*). The components are agglomerated by adding drinking water and subjected to physical treatments such as cooking and drying.[3,4] The chemical composition of couscous was reported in many studies. The importance of couscous comes from its rich complex mixture composition including carbohydrates (65 to 75 g *per* 100 g), lipids (1.50 to 3.50 g *per* 100 g), proteins (12.50 to13.50 g *per* 100 g), fibres (7 to 9 g *per* 100 g), moisture (< 13.50 %), and essential minerals (< 0.05 g *per* 100 g).[5] Couscous is one of the most ancient foods, and was developed by the indigenous inhabitants (Berbers) of North Africa. Algeria has a culinary heritage that revolves around several traditional dishes. Today, couscous, the traditional Algerian dish, is part of the world's culinary heritage. It is prepared with vegetables (carrots, green beans, zucchini, potatoes, turnips, chard, cabbage, tomatoes, onion and garlic are added to the sauce as spice), pulses, and different types of meat (red meat, chicken meat and fish).[6]

Phenolic components are commonly known as plant secondary metabolites and produced under normal and stress conditions.[7] More than 8000 phenolic compounds as naturally occurring substances have been reported.[8] These molecules prevent various diseases and possess diverse biological activities such as antioxidative, anti-inflammatory,[9] antibacterial,[10] cardioprotective potencies,[11] immune system promoting, skin protection from UV radiation, and are interesting candidates for pharmaceutical and medical application.[12]

In the scientific literature, several studies have been conducted on the physicochemical and nutritional properties of couscous. Different ingredients were used for the enrichment of couscous, such as pseudocereals (amaranth, buckwheat and quinoa), [13] bulgur flour, [5] chickpea, [14] buckwheat and legume flour [15] and soy flour and oat flour.[16]

Issoufer is a traditional preparation of 16 herbs used in the folk medicine of Algerian Sahara regions. This mixture of herbs, mostly used to enrich couscous, is commonly consumed by the local population, and intended as a remedy for women after childbirth to fight various infectious diseases (gastrointestinal and urinary tract pathogenic infections). The purpose of this study was to elucidate the effect of the enriched couscous with Issoufer powder on physicochemical, technological, and microbiological characteristics, as well as on the therapeutic (antioxidant and antibacterial) potencies.

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2 Experimental

2.1 Raw material

The plant material used in this study was a powder called Issoufer, used in the Algerian Sahara regions to enrich couscous. The powder was prepared by mixing 16 traditional plant medicines (Table 1). All plant medicines composing the Issoufer powder were well selected, weighed, and mixed (Fig. 1).

Fig. 1 – Issoufer powder

Table 1 – Composition of Issoufer powder

Compounds	Botanical name	Contents /%
Dried leaves of thyme	*Thymus* vulgaris	15.39
Dried leaves of pennyroyal	*Mentha pulegium*	15.39
Coriander seeds	*Coriandrum sativum*	7.70
Fenugreek seeds	*Trigonella foenum-gracum*	11.53
Dried leaves of parsley	*Petroselinum crispum*	7.70
Cumin	*Cuminum cyminum*	4.62
Anise *flowers*	*Pimpinella anisum*	4.62
Dried leaves of fennel	*Foeniculum vulgare*	4.62
Caraway seeds	*Carum carvi*	4.62
Dried peel of grapefruit	*Citrus maxima*	7.69
Dried leaves of basil	*Ocimum basilicum*	4.62
Bulbs of garlic	*Allium sativum*	3.84
Barley seeds	*Hordeum vulgare*	1.53
Lentil seeds	*Lens culinaris*	1.53
Currant	*Solanum pimpinelli folium*	1.53
Dried leaves of aurone	*Artemisia campestris*	3.07

2.2 Couscous preparation

The couscous was prepared according to the customary procedure of traditional couscous making.[4] Three wheat couscous were enriched with 10, 20, and 30 % of Issoufer powder, and then compared with a control couscous made 100 % of semolina (Figs. 2 and 3).

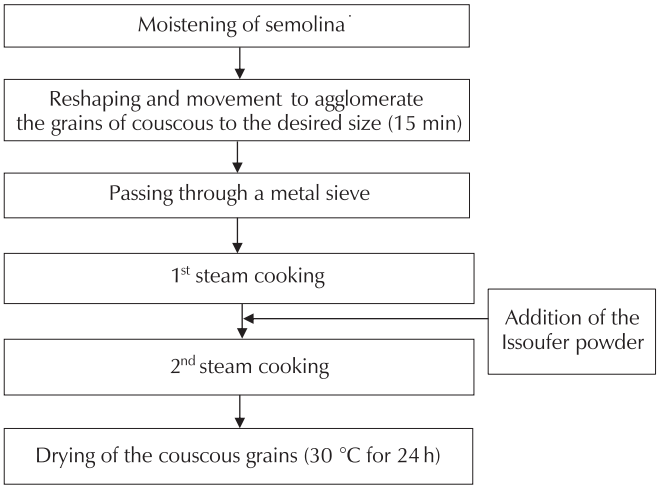

Fig. 2 – Artisanal fabrication of couscous

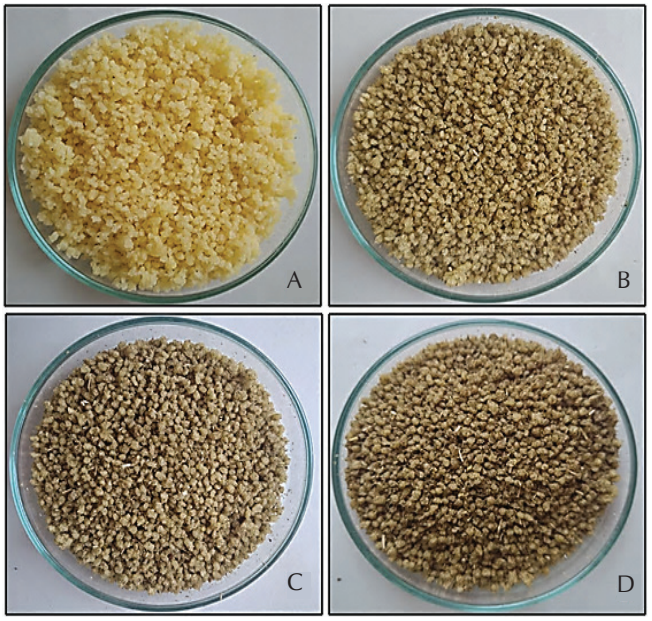

Fig. 3 – Different couscous: A) non-enriched couscous, B) couscous enriched with 10 % of Issoufer, C) couscous enriched with 20 % of Issoufer, and D) couscous enriched with 30 % of Issoufer

2.3 Determination of physicochemical properties

The samples (Issoufer powder, control, and enriched couscous) were analysed on dry weight basis (couscous grains after drying at 30 °C for 24 h) for their physicochemical composition.[17] The pH-value of the supernatant (10 g of each sample was added to 22.50 ml of water) was measured using a pH meter (Hanna instruments, Portugal). For moisture determination, 2 g of each sample were ov-

en-dried to constant weight at 105 °C for 24 h. The ash levels were determined by incineration in a muffle furnace (CONTROLS S.p.A. France) at 900 °C for 2 h. Soxhlet extraction in ethyl ether was used to quantify the total lipid. The procedure by *Lowry et al.*[18] was used for protein quantification, and the levels were given as milligram bovine serum albumin equivalents *per* gram (mg BSA Eq g^{-1}). Total carbohydrates levels were calculated by the difference: 100 % − (moisture + crude ash + total lipid + crude proteins).[19]

2.4 Microbiological properties

Microbiological analyses were intended to verify the hygienic quality of the product in order to avoid any risk to consumer health. The enriched couscous safety was assessed by microbiological analyses for total aerobic mesophilic flora, yeasts, molds, and sulphite-reducing *Clostridium*.[20]

2.5 Biological potencies of the enriched couscous

2.5.1 Preparation of the extracts of enriched couscous

Ten grams of each sample (Issoufer powder, control, and enriched couscous) was added to 100 ml of ethanol (96 %). After 24 h of contact under continuous shaking (WIS-10, Daihan Scientific Co. Ltd., Republic of Korea), the mixtures were filtered. The filtrates were then concentrated using rotary vacuum evaporator (Büchi, Switzerland) at 40 °C. The concentrated extracts, which had a viscous aspect, were stored at 4 °C in dark glass bottles until use.[21]

2.5.2 In vivo studies

Animals

Twenty-five male *Swiss albino* mice (25 and 30 g, 8 weeks old) were obtained from the Institute Pasteur (Algiers, Algeria). All the mice were housed in controlled environmental conditions, photoperiod (12 h light, 12 h dark) and temperature (24 ± 2 °C). The mice were acclimatised to environmental conditions for 2 days and had free access to food and water.[22]

Ethics

All the experiments on animals were confirmed and approved by the Department of Nutrition and Food Sciences, Faculty of Life and Natural Sciences, Hassiba Benbouali University (Chlef, Algeria). The experiments were conducted according to the guidelines and the recommendation of the "Guide for the Care and Use of Laboratory Animals".

Acute toxicity

The animals were kept fasting overnight providing only water, and the extract of powder Issoufer was given orally at the dose level of 100, 250, 500, and 750 mg kg^{-1} body weight. The animals were observed continuously for their behaviour for the first 4 h, and for mortality at the end of fitfh day.[23]

2.5.3 Bioactive content

Measurement of total phenolic content (TPC)

An amount of 200 µl of each extract, 1.50 ml Folin-Ciocalteu reagent (1 : 10) and 1.50 ml of sodium bicarbonate (60 g l^{-1}) were mixed. After 90 min, the absorbance of different extracts of couscous and the Issoufer powder was measured spectrophotometrically (Optizen 2120, Mecasys Co. Ltd., Korea) at 725 nm. The amounts of TPC are given as milligram gallic acid equivalents *per* gram of extract (mg GAE g^{-1}).[24]

Measurement of total flavonoid content (TFC)

One millilitre of each extract, 4 ml of distilled water, and 0.30 ml of sodium nitrite (5 %) were mixed. After 5 min, 0.30 ml of aluminium chloride (10 %), 2 ml of sodium hydroxide (4 %), and 2.40 ml of distilled water were added to the mixture. After 5 min, the absorbance was measured spectrophotometrically at 510 nm. The levels of TFC of different extracts (enriched couscous and Issoufer powder) are given as milligram quercetin equivalents *per* gram of extract (mg QEQ g^{-1}).[25]

2.5.4 In vitro antioxidant potencies

β-Carotene-linoleic assay

One millilitre of 3 mg of β-carotene dissolved in 30 ml of chloroform was mixed with 40 mg of linoleic acid and 400 mg of Tween® 40 (Sigma-Aldrich GmbH, Germany). After removal of chloroform at 40 °C, 100 ml of distilled water was added to the mixture. Four millilitres of the resulting emulsion was added to 200 µl of the test samples (500 µg ml^{-1}). The absorbance was recorded at 470 nm. The antiradical activity (AA) of the test samples was calculated using Eq. (1).

$$AA = ((A_{t0} - A_{t60})/A_{t0}) \cdot 100\ \% \quad (1)$$

where A_{t0} and A_{t60} are the absorbance measured at times zero and 60 min of the incubation for extracts, respectively.[26]

DPPH radical-scavenging

The mixtures containing 60 µl of extract of the enriched couscous, the Issoufer powder at a concentration of 500 µg ml^{-1}, and 1.5 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.004 %) were incubated for 30 min. The discoloration was recorded at 517 nm. The inhibition (I) was calculated using Eq. (2).

$$I = ((A_0 - A_1) / A_0) \cdot 100\ \% \quad (2)$$

where A_0 and A_1 are the absorbance of the blank and the extracts, respectively.[27]

2.5.5 Antibacterial activities

Bacterial strains

The bacterial strains used to evaluate the antibacterial properties of the different extracts of the enriched couscous and the Issoufer powder include six Gram-positive (*Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC11778, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC19115, *Micrococcus luteus* ATCC 9341, and *Staphylococcus aureus* ATCC 25923), and two Gram-negative (*Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC700603). The strains were kindly obtained from the Laboratory of Microbiology of the Faculty of Natural and Life Sciences, Abu Bekr Belkaid University (Tlemcen, Algeria). All bacteria were grown on nutrient agar (Oxoid, England) at 4 °C. The bacterial strains were reidentified on the basis of their morphologic, cultural, and biochemical characteristics.

Measurement of antibacterial activity

The antibacterial activities of the ethanolic extracts of the enriched couscous and the Issoufer powder were determined using the disk diffusion method.[28] Muller Hinton Agar was used as media. The bacterial strains were cultured in nutrient broth for 24 h and diluted with sterilised peptone water. Suspensions of tested microorganisms (0.5 McFarland units) were spread into media plates. Sterile filter paper discs of 6 mm in diameter (Oxoid, England) were impregnated with 50 µl of each extract (10 mg ml^{-1}) as well as with ethanol as negative control. The inoculated plates were incubated (Memmert GmbH + Co.KG, Germany) at 37 °C for 24 h. Antibacterial activity was determined based on the diameter measured in millimetres of the clear zone surrounding the paper discs. All the tests were performed in triplicate.

2.6 Statistical analysis

All results are given as mean ± standard deviation of three repetitions. The analysis of variance (ANOVA) was used to determine the statistical comparisons among multiple groups. The significance level (Tukey's HSD test) was accepted at $p < 0.05$.

3 Results and discussion

3.1 Physicochemical properties

The values of the physicochemical parameters of the enriched couscous with the different concentrations of Issoufer and the control couscous were variable. The addition of the powder induces a significant change in several physicochemical parameters. It was observed that Issoufer provides and contains many health-promoting molecules such as carbohydrates, minerals, and proteins (Table 2).

The moisture of the different samples (Issoufer powder, control, and enriched couscous) varied from 8.30 ± 0.55 to 12.63 ± 0.43 g *per* 100 g. As shown in Table 2, moisture values increased in enriched couscous. The moisture content of the control couscous (12.63 ± 0.43 g *per* 100 g) was significantly higher ($p < 0.05$) than that of the couscous enriched with 10 % (10.80 ± 0.18 g *per* 100 g). In contrast, couscous enriched with 20 % (9.44 ± 0.02 g *per* 100 g) and 30 % (9.12 ± 0.16 g *per* 100 g) of Issoufer powder showed the lowest moisture (Table 2).

The moisture level reduces the risk of damage during packaging and storage, and extends the shelf life of enriched couscous. Indeed, this parameter is an essential factor in the growth of microorganisms. To prevent caking and deterioration of products in storage conditions, the moisture must be lower than 12.50 %.[29]

Ash content of the different samples ranged from 1.45 ± 0.45 to 2.53 ± 0.60 g *per* 100 g. These contents were not significantly different. According to the results, the addition of powder does not improve ash levels in enriched couscous. The increase in ash content implies an increase in mineral composition, which is important for

Table 2 – Physicochemical composition of Issoufer powder, control, and the enriched couscous

	Moisture / g *per* 100 g	Ash / g *per* 100 g	pH-value	Protein / mg BSA Eq g^{-1}	Total lipids / g *per* 100 g	Total carbohydrates / g *per* 100 g
Issoufer	7.70 ± 0.55^{d}	2.53 ± 0.60^{a}	5.53 ± 0.02^{c}	10.00 ± 0.94^{c}	6.06 ± 0.11^{a}	82.65 ± 0.19^{b}
CNE	12.30 ± 0.43^{a}	1.05 ± 0.45^{a}	6.71 ± 0.02^{a}	11.80 ± 0.04^{b,c}	1.06 ± 0.23^{c}	84.21 ± 0.21^{c}
CE 10	10.50 ± 0.18^{b}	1.86 ± 0.20^{a}	5.83 ± 0.08^{b}	12.10 ± 0.02^{b}	1.14 ± 0.06^{b}	85.44 ± 0.53^{b}
CE 20	9.41 ± 0.02^{c}	2.14 ± 0.20^{a}	5.79 ± 0.01^{b}	12.50 ± 0.07^{a,b}	1.33 ± 0.02^{b}	86.02 ± 0.31^{a}
CE 30	8.99 ± 0.16^{c}	2.45 ± 0.30^{a}	5.74 ± 0.01^{b}	13.10 ± 0.23^{a}	1.53 ± 0.04^{b}	85.61 ± 0.81^{b}

CNE: non-enriched couscous, CE 10: couscous enriched with 10 %, CE 20: couscous enriched with 20 %, and CE 30: couscous enriched with 30 %. Values in the same column sharing different letters are significantly different ($p < 0.05$).

improving the nutritive quality of the enriched couscous, and could be beneficial for many people having an insufficient intake of mineral elements.[30]

The protein content of samples ranged from 10.00 ± 0.94 to 13.10 ± 0.23 mg BSA Eq g^{-1}. Couscous enriched with 30 % of the powder showed the highest content (13.10 ± 0.23 mg BSA Eq g^{-1}) followed by those enriched with 20 % (12.50 ± 0.07 mg BSA Eq g^{-1}), and 10 % (12.10 ± 0.02 mg BSA Eq g^{-1}). Based on these results, the addition of the powder slightly increased the protein content in the couscous (Table 2).

Increased protein is important for the improvement of the nutritive quality of enriched couscous. High-protein diets have beneficial roles in human health. Proteins take part in essential functions of organisms.[31]

The lipid content of Issoufer powder (6.06 ± 0.11 g *per* 100 g) was significantly higher ($p < 0.05$) than in other couscous samples. It appears from the results that the addition of the powder allowed significant elevation of fat content ($p < 0.05$) in couscous enriched with 10 % (1.14 ± 0.06 g *per* 100 g), 20 % (1.33 ± 0.02 g *per* 100 g), and 30 % (1.53 ± 0.04 g *per* 100 g), compared to the control couscous (1.06 ± 0.23 g *per* 100 g) (Table 2).

The results of this study also suggest the benefits of nutrients in Issoufer powder. The lower level of fat in the enriched couscous is desirable for many consumers, since it may reduce atherosclerosis, coronary heart disease, and obesity.

Total carbohydrates content in the enriched couscous was significantly higher ($p < 0.05$) than that of control couscous. There was a gradual increase in carbohydrates content in couscous enriched with 10 % (85.44 ± 0.53 g *per* 100 g), 20 % (86.02 ± 0.31 g *per* 100 g), and 30 % (85.61 ± 0.81 g *per* 100 g) (Table 2).

Carbohydrates are biological macromolecules and essential elements of body functions including providing energy, cellular communication, and construction.[32] Issoufer powder is composed of traditional plant medicines, which are rich in dietary fibres. Several studies have shown the health benefits of fibres. Increased fibre in a diet is correlates with the prevention of intestinal cancer, relief of constipation, and regulation of blood sugar levels, as well as reduced risk of heart disease.[33]

3.1 Microbiological analysis

The results indicated that all the couscous samples were microbiologically safe. After incubation, microbiological assessment showed the complete absence of total aerobic me sophilic flora, yeasts, moulds, and sulphite-reducing *Clostridium* colonies for Issoufer powder, control couscous, and enriched couscous. The results proved that the enriched couscous were not favourable substrates for microorganism development; this could be justified by their lower moisture (< 10.50 %).

Benayad et al.[2] studied the effect of the addition of three lentil semolina ratios (25, 50, and 75 %) on the microbiological quality of couscous. They observed less aerobic total mesophilic flora, and a total absence of yeasts and moulds for lentil couscous than for wheat couscous, which is also in agreement with our study.

3.2 Acute lethal toxicity of extract of enriched couscous

The extract of Issoufer did not change the normal behaviour of mice treated and failed to cause mortality even at the highest dose of 750 mg kg^{-1}. Acute toxicity studies showed that traditional plant medicines (Issoufer) used in the Algerian Sahara to enrich couscous were found to be safe.

Various studies have shown that Issoufer powder species are not toxic. The administration of coriander (*Coriandrum sativum*),[34] anise flowers (*Pimpinella anisum*),[35] and parsley (*Petroselinum crispum*)[36] extracts had no negative impact on metabolism. Similarly, the oral gavage of aqueous extract of *Mentha pulegium* at single doses of 0.50, 1.00, 2.50, and 5.00 mg kg^{-1}/day produced no evidence of toxicity or mortality in all mice treated during fourteen days of observation period.[37] Oral administration (100 and 200 mg kg^{-1}/day) of the methanolic extract of *Cuminum cyminum* caused no alterations in the body weight in a sample of male albino rats for an observation period of 60 days.[38] The butanolic fraction of *Ocimum basilicum* was not toxic at doses tested on male reproduction, but, instead, showed fertility improvement effects that were better with the butanolic fraction.[39]

The extracts of *Thymus vulgaris* leaves and the ripe fruits of *Carum carvi* L. showed no signs of acute toxicity, even at the highest dose tested.[40,41] The evaluation of toxicological effects of aqueous garlic bulbs (*Allium sativum*) [42] and ethanolic fenugreek seeds (*Trigonella foenumgraecum* L.) [43] extracts revealed no mortality or changing in biochemical and hematological parameters of organ function.

3.3 Bioactive content

3.3.1 Total phenolic content

The TPC of the extracts varied from 0.24 ± 0.14 to 62.07 ± 0.95 mg GAE g^{-1}. The TPC of the powder of Issoufer extract was significantly ($p < 0.05$) greater than those of the other extracts: couscous extract enriched with 30 % (15.62 ± 0.94 mg GAE g^{-1}), 20 % (7.04 ± 0.67 GAE g^{-1}) enriched couscous extract powder, and couscous extract enriched with 10 % (2.28 ± 0.48 mg GAE g^{-1}). In contrast, the low content of total phenolic compounds was found in control couscous extract (0.24 ± 0.14 mg GAE g^{-1}) (Table 3).

The TPC of methanol, acetone, and ethyl acetate extracts of the seeds of *Pimpinella anisum* L. using the spectrophotometric method were 344.10, 174.60, and 143.58 mg GAE g^{-1} dried extract, respectively.[44] The phenolic content of extract of *Mentha pulegium* was 98.33 mg GAE g^{-1}.[37] The quantitative estimate of total polyphenols showed that Fenugreek seeds extracts (*Trigonella foenum-graecum*) were rich in these compounds

(28.63 μg GAE mg^{-1} of extract).[45] The extraction with acetone (80 %) of cumin (*Cuminum cyminum*) led to elevated levels of polyphenol (18.60 mg GAE g^{-1}).[46] The content of phenolic compounds in lentils were in the range of 1.37 to 5.53 GAE g^{-1}.[47]

3.3.2 Total flavonoid content

Flavonoid levels of ethanolic extracts of samples ranged from 17.89 ± 0.91 to 0.07 ± 0.76 mg EQ g^{-1}. The TFC of Issoufer powder was significantly ($p < 0.05$) the highest (4.23 ± 0.29 mg EQ g^{-1}), followed by couscous enriched with 30 % (6.75 ± 0.77 mg EQ g^{-1}), couscous enriched with 20 % (3.54 ± 0.12 mg EQ g^{-1}), and couscous enriched with 10 % (2.08 ± 0.52 mg EQ g^{-1}). The control couscous recorded the lowest flavonoid content (0.07 ± 0.07 mg EQ g^{-1}) (Table 3).

The flavonoid content of methanol, acetone, and ethyl acetate extracts of the seeds of *Pimpinella anisum* L. was 314.68, 122.00, and 118.65 mg EQ g^{-1} dried extract, respectively.[44] The flavonoid content of mint pulley extract (*Mentha pulegium*) was 0.58 mg EQ g^{-1}.[37] Quantitative estimates of flavonoids showed that fenugreek extract (*Trigonella foenum-graecum*) was rich in these compounds (272.64 ± 0.09 μg EQ mg^{-1}).[45]

The extraction with acetone (80 %) of cumin (*Cuminum cyminum*) led to high levels of flavonoids (5.91 mg EQ g^{-1}).[46] The content of flavonoids in lentils was in the range of 0.01 ± 0.00 to 0.80 ± 0.04 mg EQ g^{-1}.[47]

Numerous studies have shown that flavonoids show a very strong ability to block the proliferation of human cells from breast, prostate, colon, lungs, and skin cancer as well as prevention against cardiovascular diseases.[48]

3.4 In vitro antioxidant potencies

3.4.1 ß-Carotene-linoleic assay

The percentage inhibition of sample extracts varied from 29.5 ± 0.98 to 97.87 ± 0.29 %. At the concentration of 500 μg ml^{-1}, the ethanolic extract of the Issoufer powder showed a percentage of antioxidant activity (97.87 ± 0.29 %) significantly higher ($p < 0.05$) than found in the other extracts tested, followed by couscous enriched with 30 % (81.96 ± 0.18 %), couscous enriched with 20 % (78.8 ± 0.34 %), and couscous enriched with 10 % (50.80 ± 0.28 %). In contrast, the control couscous showed the lowest antioxidant activity (29.5 ± 0.98 %) (Table 3).

In vitro studies have found that the antioxidant capacity of cumin (*Cuminum cyminum*) is similar to that of synthetic antioxidants, BHT and ascorbic acid.[38]

3.4.2 DPPH radical-scavenging

Percentage of the relative antioxidant activity of extracts ranged from 25.00 ± 0.28 to 97.38 ± 1.01 %. At a concentration of 500 μg ml^{-1}, the ethanolic extract of the Issoufer powder showed a significant percentage ($p < 0.05$) of antioxidant activity (97.38 ± 1.01 %), followed by the 30 % enriched couscous (80.55 ± 0.64 %), 20 % enriched couscous (79.60 ± 0.63 %) and 10 % enriched couscous (51.80 ± 0.32 %), whilst the control couscous had the lowest percentage of relative antioxidant activity (25.00 ± 0.28 %) (Table 3).

The extract of *Mentha pulegium* showed significant antioxidant activity compared to ascorbic acid and Trolox with an IC_{50} of 58.27 ± 2.72 μg ml^{-1}.[49] The antioxidant activity of fenugreek (*Trigonella foenum-graecum*) extract by DPPH showed a high antioxidant potential compared to that of BHT with IC_{50} of 5.70, 1.20, and 18.90 μg ml^{-1}, respectively.[46] The ethanolic extract of parsley leaves (*Petroselinum crispum*) showed an excellent inhibitory activity of the DPPH radical with an inhibition percentage exceeding 97 % for the concentration of 2.88 mg ml^{-1} followed by the ethanolic extract of the seeds with an inhibition rate of 75 % at the concentration of 2.52 mg ml^{-1}. In contrast, ethanolic extract from stems recorded the lowest inhibition (51.85 %) of the DPPH radical at a similar concentration.[50]

3.5 Antibacterial activity

The addition of the powder to the couscous showed a significant antibacterial spectrum compared to that non-enriched. On the other hand, no significant difference in

Table 3 – Bioactive content and antioxidant activity of Issoufer and enriched couscous

	TPC / mg GAE g^{-1}	TFC / mg QE g^{-1}	β-Carotene bleaching assay / %	I DPPH / %
Issoufer	62.07 ± 0.95^a	17.89 ± 0.91^a	97.87 ± 0.29^a	97.38 ± 1.01^a
CNE	0.24 ± 0.41^e	0.07 ± 0.07^e	29.50 ± 0.98^e	25.00 ± 0.28^d
CE 10	2.28 ± 0.48^d	2.08 ± 0.52^d	50.80 ± 0.28^d	51.16 ± 0.32^c
CE 20	7.04 ± 0.67^c	3.54 ± 0.12^c	78.89 ± 0.34^c	79.67 ± 0.63^b
CE 30	15.62 ± 0.94^b	6.75 ± 0.77^b	81.96 ± 0.18	80.55 ± 0.64^b

CNE: non-enriched couscous, CE 10: couscous enriched with 10 %, CE 20: couscous enriched with 20 %, and CE 30: couscous enriched with 30 %. TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl. Values in the same column sharing different letters are significantly different ($p < 0.05$).

Table 4 – Diameters of inhibition zones of Issoufer and enriched couscous extracts

	Issoufer	CNE	CE 10	CE 20	CE 30
Bacillus subtilis ATCC6633	29.90 ± 0.60	19.30 ± 0.03	19.20 ± 0.30	18.60 ± 0.65	17.10 ± 0.46
Bacillus cereus ATCC11778	10.40 ± 0.44	00.00 ± 0.00	7.50 ± 0.14	8.00 ± 0.48	9.50 ± 0.60
Enterococcus faecalis ATCC 29212	16.80 ± 0.94	00.00 ± 0.00	11.00 ± 0.02	14.00 ± 0.06	15.50 ± 0.23
Klebsiella pneumoniae ATCC700603	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Listeria monocytogenes ATCC19115	00.00 ± 0.00	00.00 ± 0.00	6.00 ± 0.00	6.20 ± 0.06	00.00 ± 0.00
Micrococcus luteus ATCC 9341	12.00 ± 0.29	00.00 ± 0.00	9.50 ± 0.28	9.00 ± 0.34	10.50 ± 0.18
Staphylococcus aureus ATCC 25923	16.50 ± 0.43	00.00 ± 0.00	13.00 ± 0.02	8.00 ± 0.01	9.00 ± 0.01
Pseudomonas aeruginosa ATCC 27853	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00

CNE: non-enriched couscous, CE 10: couscous enriched with 10 %, CE 20: couscous enriched with 20 %, and CE 30: couscous enriched with 30 %.

antibacterial effect was recorded between couscouses enriched with the different mass ratios of Issoufer powder. The large inhibition zones were observed in *Bacillus subtilis* ATCC6633 (29.90 ± 0.60 mm) with the ethanolic extract of the powder. *Bacillus subtilis* ATCC6633 and *Enterococcus faecalis* ATCC 29212 were the most sensitive bacteria to the effect of the extracts, unlike Gram-negative bacteria: *Klebsiella pneumoniae* ATCC700603 and *Listeria monocytogenes* ATCC19115, which were the most resistant. The ethanolic extract of the control couscous was inactive against the pathogenic bacteria tested, with the exception of *Bacillus subtilis* ATCC6633 where it exerted a moderate activity with an inhibition zone diameter of 19.30 ± 0.03 mm (Table 4).

Jebali et al.[51] tested the antibacterial activity of the methanolic extracts of the leaves of *Mentha pulegium* L. collected from Tunisian regions (Bizerte and Kef) against *Bacillus subtilis*. They found inhibition zone diameters of 31.00 ± 0.33 and 29.00 ± 0.30 mm, respectively. These values are significantly important compared to that of gentamicin (25.00 ± 0.00 mm) used as positive control (antibiotic). The aqueous fenugreek extract (*Trigonella foenum-graecum*) showed antibacterial activity against Gram-positive bacteria, while there were no obvious effects on Gram-negative bacteria.[45]

Extracts from the leaves and stems of *Petroselinum crispum* have antibacterial activities against *Bacillus subtilis* and *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus pyogenes*.[52] The essential oil of fennel seed (*Foeniculum vulgare*) possessed antibacterial activity against *Staphylococcus albus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Shigella dysenteriae*, and *Escherichia coli*.[53] Garlic extract (*Allium sativum*) demonstrated dose-dependent antimicrobial activity against three reference strains of *Helicobacter* at concentrations of 2 and 5 mg ml^{-1}.[54]

The antibacterial activity of phenolic compounds is primarily due to alteration of lipid fractions in the plasma membrane of microorganisms, resulting in disruption of membrane permeability and loss of intracellular organelles.[55] Phenolic compounds act by disrupting the enzymatic mechanisms involved in energy production for both bacteria and yeasts. They can also destroy germs by modifying their structural compounds.[56] Flavonoids are known for their powerful antibacterial power. They could potentially have an effect in iron chelation, which prevents intracellular penetration of the cofactor Ca^{+2} into the bacterial cell, and inhibits their activity.[57]

The antibacterial activity of the mixture (couscous and issoufer) could be attributed to the synergistic action between compounds contained in each material.[58]

The antimicrobial mechanisms of phenolic compounds are still far from being fully understood. The diminution of antibacterial activity with increment of Issoufer concentration may be due to the antagonist action of minor compounds contained in its chemical mixture. The present research seems to be the first investigation of antibacterial activity of Issoufer; hence, further studies are suggested to define active constituents, their mechanism of action, and possible synergistic and/or antagonistic effects.

4 Conclusions

The current study researched, for the first time, the impact of fortification of couscous with the traditional preparation of medicinal plants, namely Issoufer. The results revealed that the incorporation provides a significant enhancement in several physicochemical parameters. A significant increase was observed for values of ash, proteins, lipids, carbohydrates, phenolic compounds, flavonoids, and antioxidant activity for all the couscouses enriched with the different mass ratios of Issoufer. However, moisture and pH-value showed a significant decrease in the enriched couscous as compared to control couscous. The acute toxicity study showed that the oral administration of the different doses of Issoufer extract caused no change in animal behaviour. The fortification of couscous with Issoufer provides a healthy and functional food, rich in nutrients, bioactive compounds, and improves technological and microbiological properties. This traditional preparation can also be used to improve the bioavailability of micronutrients in alimentary paste.

Conflict of interests

All authors declare no conflicts of interest.

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SAŽETAK

Poboljšanje tehnoloških svojstava i terapeutskog potencijala kus-kusa dodatkom tradicijskog preparata Issoufera: tradicionalna biljna medicina

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Obogaćivanje hrane je način da se smanji učestalost nedostataka mikronutrijenata u populaciji. Kus-kus je namirnica koja se u Alžiru konzumira u velikoj mjeri. Stoga je cilj ovog istraživanja bio dobiti obogaćeni kus-kus s poboljšanim terapeutskim potencijalom i dobrim fizikalno-kemijskim, tehnološkim i mikrobiološkim karakteristikama. Pšeničnom kus-kusu dodan je tradicionalni pripravak Issoufer (10, 20 i 30 %), te je smjesa uspoređena s kontrolnom čistom kus-kus krupicom. Znatno povećanje ($p < 0,05$) pepela, proteina, lipida, ugljikohidrata, fenolnih spojeva, flavonoida i anti-radikalne aktivnosti, zabilježeno je kod svih smjesa s dodanim Issoufer pripravkom. S druge strane, znatno ($p < 0,05$) su se smanjili udio vlage te pH-vrijednost. Studija akutne toksičnosti nije pokazala smrtonosne učinke i znakove toksičnosti pri ispitanim dozama ekstrakta Issoufera (100, 250, 500 i 750 mg kg^{-1}) tijekom pet dana promatranja. Rezultati antibakterijske aktivnosti pokazali su da su promjeri zona inhibicije dosegli vrijednost od 29,90 ± 0,60 mm. Na temelju dobivenih rezultata, Issoufer prah može se smatrati dobrim sastojkom za razvoj funkcionalnog kus-kusa prirodno obogaćenog sekundarnim metabolitima te se može upotrebljavati za prevenciju više bolesti, ali i u prehrambenoj industriji.

Ključne riječi
Obogaćivanje hrane, kus-kus, Issoufer preparat, antioksidativna aktivnost, antimikrobna aktivnost

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