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Active Components of Squid Ink and Food Applications

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IN Egypt, squid ink is disposed of during squid preparation, which may cause environmental pollution and health problems. Therefore, this study was carried out to make use of the active ingredients found in the squid ink as by-products. The chemical composition, the mineral, total phenols, and flavonoids contents of squid ink were determined. DPPH radical scavenging activity, cytotoxicity, and antitumor activity of squid ink on human tumor cell line (Lung carcinoma cell line) were also tested beside its antimicrobial activity. Two mixtures of black or brown squid ink sauce were prepared. The results indicated that the antioxidant activity was 91.66%. The results of the cell viability and toxicity assay of squid ink indicated that as the concentration of squid ink increased the viability percentages were decreased, while inhibitory activity percentages were increased. The IC_{50} [the concentration causing the death of 50% of the human tumor cell line (Lung carcinoma cell line A549)] was 22 µg/mL. The results also indicated that the squid ink had antimicrobial activity against *Bacillus subtilis, Aspergillus fumigates, Aspergillus niger, Candida albicans, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia,* and *Proteus vulgaris* with zone inhibitions of 19, 18, 12, 13, 11, 13, 12 and 10 mm, respectively. The overall acceptability scores of the black and brown squid ink sauces were 9.35 and 10, respectively. In conclusion, the formulated squid ink sauces with a strong odor, high acceptability, and different colors as well as anti-oxidant, anti-microbial and anti-cancer activities can fulfill the desires of many consumers.

Keywords: Squid ink, Anti-oxidant, Anti-microbial.

Introduction

The squid belongs to the order Teuthida, in cephalopods. It has eight arms and longer tentacles that help them to swim. It tends to stay in cold and deep water. It is black and can be found in several sizes. These species are not more than 60 cm in length. In some countries, it is known as the calamari (Scientific American studies, 2018).

Cephalopod species creates ink which helping it escape from predators. The ink is diffused like a cloud as a screen to help hide him a little. Therefore, it is necessary for the ink to have dark properties and thick stability to prevent it from disperses quickly in the surrounding water. The color of ink differs in each species of this family and is known by many names such as squid ink, cuttlefish ink, tintacalamar, hero di seppia, black squid ink, cephalopod ink, and octopus ink. Octopus ink is usually black ink, while squid color is blue-black and squid ink is like brown shade. In some countries, they prefer squid ink because of its unique appearance (Caldwell, 2005).

Squid ink is very high in several important nutrients, particularly the antioxidants, which well-known help to protect the cells and the heart

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against damage from free radicals. The noticeable blue-black color of squid ink is due to the presence of large amounts of melanin pigment. The melanin pigment is created in mature cells of the ink gland which is present in the bottom of the ink sac. Additionally, it also contains large quantities of proteins, lipids, and minerals, etc. (Palumbo et al., 1997). Squid Ink has better antimicrobial activity against various microbes, making it a great choice of natural antibiotic (Nithya et al., 2011).

Cuttlefish ink extract and freshwater mussel extract had no toxic effect on experimental rats up to a concentration of 2000 mg/kg. The LD50 in mice was therefore taken as above 2000 mg/kg. This dose was considered one-tenth of the proposed LD50, that is, 200 mg/kg body weight (Fahmy and Soliman, 2013). Cuttlefish ink is usually discarded as a processing waste but is common in the cuisine of Japan, Italy, and Spain. Surprisingly, there are few available literatures regarding its proximate composition which is necessary to ensure that it meets the nutritional requirements essential for the growth and health of a living being. Food with high protein is generally considered to be nutritionally better and hence, ink from *S. prabahari* with highest protein content is nutritionally preferred among the three cuttlefish species *Sepia pharaonis, S. prabahari* and *S. ramani* (Ganesan et al., 2017).

Loligo duvauceli ink generated as a by-product in the processing industry has large numbers of compounds like phenol, flavonoid, protein, carbohydrate, amino acids. The protein and flavonoids were present in the higher amount where as sterols, terpenoids and saponins were absent (Nisha and Suja, 2018).

Squid ink, which is discarded as a by-product, could be used as a good source for natural antioxidants after melanin removal. Melanin-free ink with high thermal stability possessed radical scavenging and metal chelating activities. After ultrafiltration, the fraction with lower molecular weight had a greater antioxidative activity (Vate and Benjakul, 2013).

Squid ink has many uses in the food industry due to its distinct flavor. Squid ink is sold in commercial markets to be used as a food flavoring (Hazan, 1992). In many dishes, squid ink is used as a food coloring, for example, black rice, baby squid in ink sauce, ink soup with pork and squid and imitation caviar (Marquinet, 2001). Squid ink is an ingredient in bread, sweets, tofu, pasta (spaghetti), curry, potato chips, sweets, snacks (Kim, 2009), sauces (Kim, 2008)

This study aimed to prepare two types of sauce that contain squid ink, which is characterized by strong odor and its anti-oxidant and anti-microbial activities as well as anti-cancer activity were also evaluated.

Materials and Methods

Materials

Squids were purchased from Alexandria seaport- Alexandria - Egypt. Fresh onion, potato, onion powder, garlic powder, ginger, salt, sugar, cane vinegar, and cumin were purchased from the local market, Cairo, Egypt. All chemicals were obtained from Sigma Chemical Co., Saint Louis, Missouri, U.S.A with a purity 96-99%.

Methods

Preparation of squid ink

Squids were put immediately in ice at the ratio of 1:1 (squid: ice w:w) then placed in an insulated icebox. Squids were dissected and the ink sacs were taken out. The surface of the ink sac was sterilized with ethanol (El Gomhoureya Company 80%). The ink duct was cut with sterile scissors and the sac was gently squeezed then the excreted ink was collected in sterile bottles. The squid ink was kept at -18 °C (Electrostar ES300 Chest Deep Freezer – 300 L, Egypt) until analysis.

Preparation of pureed potato

The potatoes were washed and peeled, then cut into small pieces. 250 g of potatoes were cooked in 500 mL of drinkable water until they became edible, then the potatoes were mashed in the cooking water.

Preparation of squid broth

After removing the ink from the squid, the squid was thoroughly washed. The washed squid was cut into small pieces, 250 g of squid pieces, 50 g of fresh onion, and 500 mL water were cooked till became edible (for about 30 min.). Then the broth was collected and used as squid broth.

Preparation of squid ink sauce

Two different squid ink sauce formulas were prepared according to Escuela De Hostelería De Leioa (2020) with some modified as follows (Table 1) black squid ink sauce (potato puree, squid broth, squid ink, onion powder, garlic powder, black pepper, ginger, cumin, salt, sugar, and cane vinegar) and brown squid ink sauce (the same previous ingredients with the addition of

paprika powder to produce brown color). Squid ink sauce was prepared by the well mixing of all ingredients of each formula separately.

Analytical methods

Chemical composition of squid ink

Proximate chemical contents of squid ink including moisture, protein, ash, fat, and minerals (iron, zinc, calcium, potassium, sodium, phosphorous, magnesium, and manganese) were determined according to the methods outlined in AOAC (2005). Carbohydrate was calculated by difference. The determination of cadmium, lead, and mercury in squid ink sample were performed according to the methods described by Haeng-Shin et al. (2006).

Phenolic and flavonoid compounds contents of squid ink

Total phenolic compounds contents of squid ink were determined calorimetrically using Folin–Ciocalteu reagent (as gallic acid equivalent) according to the method described by Singleton et al. (1999). Total flavonoid compounds were determined (as quercetin equivalent) according to the methods of Zhishen et al. (1999).

Identification of phenolic acids and flavonoid compounds of squid ink

Phenolic and flavonoid compounds were fractionated using HPLC according to the method of Goupy et al. (1999) and Mattila et al. (2000), respectively.

Antioxidant activity of squid ink

The antioxidant activity of squid ink was determined using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). Where odd electron in the DPPH free radical had a strong absorption maximum at 517 nm. The color turns from purple to yellow as the molar absorptive of the DPPH radical reduced when the odd electron becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H as mentioned by Choi (2010). The DPPH assay was measured according to the method reported by Brand-Williams et al. (1995).

Cytotoxicity and antitumor activity of squid ink on human cell lines

Measuring of cytotoxicity of the squid ink was tested according to the MTT (open form?) assay method of Mosmann (1983) using squid ink concentrations ranged between 0.78 to 100 µg/mL against the human tumor cell line (Lung carcinoma cell line A549). This cytotoxic activity test (In vitro bioassay on human tumor cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt. The mean of the cell viability values was compared to the control to determine the effect of the squid ink on cells and % of cell viability was plotted against the concentration of the squid ink. The minimum concentration of the squid ink that was toxic to Lung carcinoma cell line A549 was recorded as an effective concentration compared to a positive control (A positive control which composed of 100µg/mL was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions).

TABLE 1. Squid ink sauce formulas (Escuela De Hostelería De Leioa, 2020)

Ingredients (g)	Squid ink sauce	
	Black	Brown
Potato puree	100	100
Squid broth	340	335
Squid ink	10	10
Onion powder	2.5	2.5
Garlic powder	2.5	2.5
Black pepper	5	5
Ginger	2.5	2.5
Cumin	2.5	2.5
Salt	5	5
Sugar	15	15
Cane vinegar	15	15
Paprika powder	---	5

Percentage of viability = absorbance of the sample/absorbance of control

Percentage of toxicity = 100 ─ the percentage of viability

Antimicrobial activity of squid ink

The antimicrobial activity of squid ink was tested by the disc diffusion method according to Rios & Recio (2005).

Sensory evaluation of squid ink sauce

The sensory evaluation was carried out to get consumer response for the overall acceptability of the squid ink sauce. The sensory attributes including taste, odor, color, consistency, and overall acceptability were evaluated by 10 trained members' panelists from the staff members Food Technology Research Institute, Agricultural Research Center. Each panelist was provided with the sample in an unlabeled transparent cup under white lights and asked to cleanse the palate with water before tasting the second sample according to the method of Jayasena et al. (2008).

Statistical analysis

Means of the results are statistically analyzed using one-way analysis of variance (ANOVA), where significant differences were observed at 5% standard deviations (SD). Statistical software (Assistat Version 7.7, Brazil) was used for all statistical analyses according to Silva & Azevedo (2009).

Results and Discussion

Chemical composition of squid ink

The results presented in Table 2 showed the chemical constituents of the squid ink (on a fresh weight basis). It could be noticed that the squid ink contained a high amount of moisture (87.9%). However, the lowest contents were observed for protein, fat, ash, and carbohydrate.

These results were agreed with those reported by Ellouze et al. (2014) which found that squid ink contained moisture, protein, fat, ash, and carbohydrates by about 66.12, 18.71, 3.33, 5.24 and 10.58%, respectively. Ganesan et al. (2017) found that the moisture, protein, fat, ash, and carbohydrates were approximately (87.90, 86.75 and 87.38%), (11.12, 12.01 and 11.38%), (0.19, 0.21 and 0.20%), (0.12, 0.09 and 0.11%), and (0.62, 0.70 and 0.68%) of ink from three cuttlefish species *Sepia pharaonis, S. prabahari and S. ramani,* respectively. Also, Nadarajah et al. (2017) and Jeyasanta & Patterson (2020) found that moisture, protein, fat, ash, and carbohydrates contents of *Loligo vulgaris* and *Loligo duvauceli* inks were 79.25, 13.67, 0.91, 0.25 and 5.59%, respectively.

Minerals contents of squid ink

Mineral content was determined and the obtained results are shown in Table 3. The results indicated that squid ink contained Fe, Zn, Ca, P, K, Mg, Mn, and Na. K showed the highest value of the minerals content (24.47 ppm), followed by P (20.66 ppm) then Ca (12.88 ppm). However, the lowest value was that of Fe (0.95 ppm). The results also, showed that heavy metals contents of squid ink (cadmium, lead, and mercury) were very small and under the permeable limits according to the Egyptian Standards for heavy metals (2007). These results are in agreement with Haeng-Shin et al. (2006). These results revealed that the obtained squid ink could be considered healthy material and could be used in food products.

Phenolic and flavonoid compounds contents of squid ink and its antioxidant activity

There is a direct relationship between phenolic compounds and the levels of color pigments (Pietta, 2000 and Elham et al., 2006). The level of color pigments was found to be correlated with the antioxidant activity (Halliwell et al. (2005).

TABLE 2. Chemical composition of squid ink (on fresh weight basis).

Components	Contents (%)
Moisture	87.9a±2.55
Protein	6.0b±1.65
Fat	2.19d±0.33
Ash	0.9e±0.52
Carbohydrate	3.0c±0.18

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

TABLE 3. Minerals contents of squid ink.

Minerals	Contents (ppm)
Fe	0.95 [g] ±0.73
Zn	2.51 [d] ±0.11
Ca	12.88 [e] ±0.36
P	20.66 [b] ±0.55
K	24.47 [a] ±0.23
Mg	2.13 [e] ±0.10
Mn	2.4 [d] ±0.13
Na	1.95 [f] ±0.45
Cd	0.013 [i] ±0.03
Pb	ND*
Mercury (Hg)	0.0031 [j] ±0.07

ND*: Not detected
Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

The results presented in Table 4 showed the total phenolic and flavonoid contents of squid ink. From these results, it could be observed that the total phenolic content of squid ink was 192.03 ppm, while flavonoid contents were 3065.11 ppm. Nisha and Suja (2018) found that the total phenol contents in the methanol extract and partially purified form of *Loligo duvauceli* ink were 0.008 and 2.65 mg equivalence of gallic acid, respectively. While, flavonoid contents were 0.00224 and 1.323 mg equivalence of quercetin, respectively.

The antioxidant activity of squid ink can be attributed to the presence of polyphenols and flavonoids. The antioxidant activity of squid ink is shown in the same Table 4. The results indicated that the antioxidant activity was 91.66%. These results are consistent with the results of Fahmy and Soliman (2013) which found that cuttlefish ink extract had DPPH radicals activity ranged from 86.14 to 95.19%. Zaharah & Rabeta (2018) found that the DPPH inhibition value of the distilled water extract of the squid ink powder was 94.87%. Agustini et al. (2018) found that the diluted squid ink [10 times using cooled ionized water (4ºC)] had a higher value of free radical inhibitors than pure and diluted squid ink (5 times).

Identification of phenolic compounds of squid ink

The results presented in Table 5 showed the phenolic compounds of squid ink. The major component of the present phenolic was found to be pyrogallol at a concentration of 76.956 ppm followed by catechin (9.474 ppm) then gallic acid (6.420 ppm). However, other phenolic compounds were also found such as catechol, 3 OH-tyrosol, P-OH benzoic, vanillic, caffeine, and coumarin but in low concentration varied between 3.285 (P-OH benzoic) to 0.463 (coumarin). Phenolic compounds may play an important role in preventing the damage of the gastrointestinal tract caused by reactive species present in foods or generated within the stomach and intestines (Halliwell, 2007). Queiroz et al. (2019) reported that gallic acid has many biological activities because it is a strong antioxidant with low toxicity, therefore it used in food, pharmaceutical, and cosmetic industries for its ability to inhibit lipid peroxidation.

Identification of flavonoid compounds of squid ink

The results of the identification of flavonoid compounds of squid ink are shown in Table 6. The results presented in Table 6 indicated that apigenin 6-rhamnose-glucose was present at a high level (2136.85 ppm) followed by apigenin 7-glucose (1065.172 ppm) then hesperidin (287.326 ppm). Naringin, rutin, naringenin, rosmarinic, quercitrin, and kaempferol ranged from 16.708 ppm (naringin) to 0.536 ppm (kaempferol). Hertog et al. (1993) found that by increasing dietary intake of flavonoids, especially quercetin the incidence of coronary heart disease and stroke decreased. Queiroz et al. (2019) reported that the presence of catechin and quercetin may be led to the synthesis of dextran molecules with antioxidant

activity. Also, they found that the conjugation between dextran and gallic was more efficient as an antioxidant agent in total antioxidant capacity (13 times) and was more efficient than dextran in superoxide radical-scavenging (60 times) and reducing power assays (90 times). Sharma (2006) reported that flavonoids have many vital benefits for human health. Flavonoids possess many activities, for example, anti-oxidant and free radical scavenging, in addition to vasodilatation, and improved blood circulation for Alzheimer's patients. Flavonoids have anti-cancer, anti-tumor, and anti-microbial properties.

TABLE 4. Phenolic and flavonoid compounds contents of squid ink and its antioxidant activity.

Parameters	Value
Phenolic (ppm)	$192.03^{b}\pm6.98$
Flavonoid (ppm)	$3065.11^{a}\pm5.88$
Antioxidant activity (%)	$91.66^{c}\pm3.95$

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

TABLE 5. Identification of phenolic compounds of squid ink.

Components	Contents (ppm)
Catechol	$2.251^{f}\pm0.65$
3 oH-Tyrosol	$2.470^{e}\pm0.41$
Pyrogallol	$76.956^{a}\pm7.55$
Gallic acid	$6.420^{c}\pm1.00$
Catechin	$9.474^{b}\pm2.30$
p-oH benzoic	$3.285^{d}\pm0.55$
Vanillic	$1.336^{g}\pm0.33$
Caffeine	$1.048^{h}\pm0.24$
Coumarin	$0.463^{i}\pm0.41$

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

TABLE 6. Identification of flavonoid compounds of squid ink.

Components	Contents (ppm)
Naringin	$16.708^{d}\pm2.65$
Rosmarinic	$2.518^{f}\pm1.05$
Apigenin 6-rhamnose -glucose	$2136.85^{a}\pm5.87$
Hesperidin	$287.326^{c}\pm8.44$
Rutin	$15.554^{d}\pm1.92$
Qurectrin	$1.729^{g}\pm0.22$
Naringenin	$3.577^{e}\pm0.55$
Kaempferol	$0.536^{h}\pm0.11$
Apigenin7-glucose	$1065.172^{b}\pm5.77$

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

Cytotoxicity of squid ink

Cytotoxicity analysis was carried out to verify if squid ink is able to produce a lethal effect on the human tumor cell line (Lung carcinoma cell line A549). Cytotoxicity of squid ink against the human tumor cell line (Lung carcinoma cell line A549) was studied using different preparations with concentrations varied between 0.00 to 100 µg/mL. The results of cell viability and toxicity assay of squid ink are shown in Tables 7 and 8. These results indicated that as the concentration of squid ink increased the viability percentages were decreased, while inhibitory activity percentages were increased. Decreasing in the squid ink concentration from 100 µg/mL to 0.78 µg/mL led to an increase in the viability percentage from 23% to 91% and to decrease the inhibitory activity percentages from 77% to 9%. As indicated in Table 8 it was found that the maximum percentage of inhibitory (70 %) was observed with the maximum concentration (100 µg/mL). Moreover, the IC_{50} [the concentration causing the death of 50% of the human tumor cell line (Lung carcinoma cell line A549)] was found to equal 22 µg/mL. These results emphasized the previous results found by Ellouze et al. (2014) where they evaluated the role of cuttlefish ink of *Sepia officinalis* on viability and proliferation of human glioblastoma cells U87. They found a cytotoxic effect of cuttlefish ink at increasing doses with a concentration higher than 20µg/mL. They also reported that the IC_{50} value was 25µg/mL which may be due to tyrosinase, which responsible for the cephalopod ink toxic. Diaz et al. (2014) reported that the crude and partially purified squid inks were having good anti-carcinogenic activity on the HepG2 cell line. They found that the IC_{50} value was obtained at a concentration of 125 µg. Fahmy & Soliman (2013) found that cuttlefish (*Sepia officinalis*) ink extract has cytotoxic activity against hepatocellular carcinoma (HepG2) cell line. They reported that the IC_{50} of *Sepia officinalis*, ink extract was 76 µg/mL.

TABLE 7. Cell viability assay.

Squid ink conc. (µg/mL)	Viability (%)
100	23^{j}±4.75
75	28^{i}±3.33
50	34^{h}±3.63
25	49^{g}±2.55
12.5	60^{f}±1.33
6.25	64^{e}±2.84
3.125	73^{d}±1.43
1.56	82^{c}±2.99
0.78	91^{b}±2.35
0	100^{a}±4.55

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

TABLE 8. Evaluation of anticancer activity of squid ink (cytotoxic effect) against human tumor cell line (Lung carcinoma cell line A549).

Squid ink conc. (µg/mL)	Inhibitory (%)
100	77^{a} ±2.55
75	72^{b} ±3.92
50	66^{c} ±3.65
25	51^{d} ±2.55
12.5	40^{e} ±1.74
6.25	36^{f} ±2.14
3.125	27^{g} ±3.00
1.56	18^{h}±1.99
0.78	9^{i} ±3.57
0	0
IC_{50}	22±2.95

IC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs

Values are means of 3 replicates ± SD, numbers in the same column, followed by the same letter, are not significantly different at 0.05 level.

Antimicrobial activity of squid ink

Antimicrobial activity of squid ink was studied against some of the fungi and bacteria strains. The results presented in Table 9 indicated that the maximum antimicrobial activity was found with *Bacillus subtilis* (RCMB 015 (1) NRRL B-543) with zone inhibitor of 19 mm followed by *Aspergillus fumigates* (RCMB 002008) which showed 18 mm zone inhibitor. However, the other strains showed zone inhibitor varied between 10 mm [*Proteus vulgaris* (RCMB004 (1) ATCC 13315] to 13 mm [*Candida albicans* (RCMB 005003 (1) ATCC 10231)]. The obtained results are harmony with the results of Nadarajah et al. (2017). They found that the inhibition zones of *Escherichia coli, Klebsiella pneumonia,* and *Staphylococcus aureus* which affected by *Loligo vulgaris* ink were 14, 13, and 12mm, respectively. Pangemanan & Mintjelungan (2020) found that the concentration of 1.56% of the squid ink extracted from *Loligo sp.* was the minimum inhibitory concentration on the growth of *Staphylococcus aureus* and *Streptococcus mutants*. Jeyasanta & Patterson (2020) found that the methanolic extract of *Loligo duvauceli* has antibacterial activity against three pathogens bacteria (*E. coli*, *Klebsiella pneumonia,* and *Pseudomonas aeruginosa*). They found that the inhibition zones were 21, 23 and 20 mm, respectively. These results are more than our results, it may be due to the different squid type and the method of ink preparation.

Sensory evaluation of squid ink sauce

The black and brown squid ink sauces were subjected to sensory evaluation for its taste, odor, color, consistency, and overall acceptability. The results are shown in Table 10. Samples were given to the panelists, and they were asked about their acceptance and satisfaction with the sauces under study. No significant differences were found between the two sauces for odor, consistency, and overall acceptability ($p>0.05$), while taste and color were significantly different between the black and brown squid ink sauces ($p<0.05$). This may be due to the presence of paprika powder. This difference in color may be used to meet the wishes of a large number of consumers especially people who do not prefer black color. The overall acceptability scores of the black and brown squid ink sauces were 9.35 and 10, respectively. The great acceptability of the black squid ink sauce indicated that the addition of paprika powder led to change the color but not affected on overall acceptability.

TABLE 9. Antimicrobial activity of squid ink.

Microorganisms		Zone inhibitor (mm)
Fungi	***Aspergillus fumigates* (RCMB 002008)**	18
	***Aspergillus niger* (RCMB 002005)**	12
	***Candida albicans* RCMB 005003 (1) ATCC 10231**	13
Gram Positive Bacteria	***Staphylococcus aureus* (RCMB010010)**	11
	***Bacillus subtilis* RCMB 015 (1)NRRL B-543**	19
Gram Negatvie Bacteria	***Escherichia coli* (RCMB 010052)ATCC 25955**	13
	***Klebsiella pneumonia* RCMB003(1)ATCC13883**	12
	***Proteus vulgaris* RCMB 004 (1) ATCC 13315**	10

TABLE 10. Sensory evaluation of squid ink sauce.

Parameter (10)	Squid ink sauce	
	Black	**Brown**
Taste	$8.63^{b} \pm 0.02$	$9.72^{a} \pm 0.04$
Odor	$10.00^{a} \pm 0.01$	$9.65^{a} \pm 0.04$
Color	$7.55^{b} \pm 0.05$	$9.00^{a} \pm 0.03$
Consistency	$9.35^{a} \pm 0.01$	$9.55^{a} \pm 0.04$
Overall acceptability	$9.35^{a} \pm 0.01$	$10.00^{a} \pm 0.06$

Values are mean of three replicates ± SD, number in the same row followed by the same letter is not significantly different at 0.05 level

From the previous results, it could be revealed that squid sauce had benefit characteristics related to its antioxidant component content, anticancer effect, and antimicrobial effect, so it could be utilized in the food preparations. However, more experiments must be performed to study their effect on the shelf life of foods.

Conclusion

This study revealed that squid ink contains essential nutrients and high mineral content (K, P, and Ca). Squid ink has many important active components such as polyphenols and flavonoids. The study also showed that squid ink has the ability to scavenge free radicals with high efficiency, which indicates that squid ink has excellent properties as an antioxidant. By measuring the ability of squid ink on the toxicity of the human tumor cell line (Lung carcinoma cell line A549), it is clear that it is able to produce a lethal effect on cancer cells. It also has a great ability to inhibit many pathogenic microbes. Also, the results indicated the possibility of the production of squid ink sauce with a high degree of acceptance.

It is noteworthy that squid sauce had benefit characteristics related to its antioxidant component content, anticancer effect, and antimicrobial effect, so it could be utilized in the food preparations. However, more experiments must be performed to study their effect on the shelf life of foods.

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المكونات النشطة لحبر الحبار واستخدامه في تطبيقات الطعام

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**قسم بحوث تكنولوجيا اللحوم والاسماك – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة – مصر

يتم في مصر التخلص من حبر الحبار أثناء تحضير الحبار ، مما قد يسبب تلوثًا بيئيًا ومشكلات صحية. لذلك أجريت هذه الدراسة بهدف الاستفادة من المكونات النشطة الموجودة في حبر الحبار كمنتجات ثانوية. تم تقدير التركيب الكيميائي ، وتحديد محتويات المعادن ، إجمالي الفينولات والفلافونويد ، وتم الكشف عن مكوناتها. تم تحديد النشاط المضاد للاكسدة بطريقة الـ DPPH. تم اختبار السمية الخلوية والنشاط المضاد للأورام لحبر الحبار على خط خلية الورم البشري (خط خلية سرطان الرئة A٥٤٩). كما تم تقدير قدرة حبر الحبار على تثبيط بعض الميكروبات المسببة للأمراض. تم تحضير خلطتين من صلصة حبر الحبار احداهما باللون الاسود للحبر والاخري باللون البني. أوضحت النتائج أن نشاط مضادات الأكسدة ٩١,٦٦٪. أشارت نتائج قياس حيوية وسمية الخلية لحبر الحبار إلى أنه مع ازدياد تركيز حبر الحبار انخفضت نسبة الحيوية بينما ازدادت نسبة النشاط السام للخلايا. دلت النتائج علي ان $IC_{٥٠}$ (التركيز الذي تسبب في وفاة ٥٠٪ من خط خلية الورم البشري) كان ٢٢ ميكروغرام / مل. أشارت النتائج أن حبر الحبار له نشاط مضاد للميكروبات المرضة الاتية *Bacillus subtilis* , *Staphylococcus aureus* , *Escherichia coli* , *Klebsiella pneumonia* , *Proteus vulgaris* , *Candida albicans* , *Aspergillus fumigates* , *Aspergillus niger* حيث كانت قطر منطقة تثبيط كل منهم كالاتي ١٩ و ١٨ و ١٢ و ١٣ و ١١ و ١٣ و ١٢ و ١٠ ملم على التوالي. كانت درجة القبول العام لصلصات حبر الحبار الأسود والبني ٩,٣٥ و ١٠ على التوالي.

من النتائج السابقة ، يمكن استنتاج أن صلصة حبر الحبار لها خصائص مفيدة تتعلق بمحتواها من مكونات مضادات الأكسدة ، مضادات السرطان ومضادات الميكروبات المرضية. لذلك يمكن استخدام حبر الحبار في المستحضرات الغذائية. ومع ذلك يجب إجراء المزيد من التجارب لدراسة تأثيرها على مدة صلاحية الأغذية.