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Impact of Bee, Palm Pollen and Wheat Germ on The Physicochemical, Functional Properties, and Free Amino Acid Profile of Spreadable Processed Cheese

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THE purpose of this paper is to produce a new spreadable processed cheese with functional and nutritional value. Bee pollen (BP), Palm pollen (PP) and Wheat germ (WG) at level 5% were used individually as a functional and natural ingredient in the production of Spreadable processed cheese to determine their effect on physicochemical, functional and sensory properties of the product.The addition of these ingredients lead to improvement of the physicochemical composition as a result of the increase in nutritional value of processed cheese in terms of proteins, carbohydrates, ash and enhancing the physical properties. Highcontent of phenolic and antioxidant activity has been observed in processed cheese containing BP, PP and WG. High values of phenolic compounds were obtained in PP-cheese which had an equivalent 1.87 mg Gallic acid/g cheese and 62.47% antioxidant activity, while the lowest ones an equivalent of 1.38 mg Gallic acid/g cheese and 51.90% antioxidant activity were observed in WG-cheese. In addition, a balanced amino acid profile with vital essential amino acids was obtained in the final processed cheese product and the PP-cheese contained a high content of these acids. Moreover, the panelists preferred the cheese samples containing these additives without negative notes, while WG-cheese had the highest scores.This ensure the acceptance of these additives as a functional component in the manufacture of processed cheese at level 5%, which opens the way for future experiments for testing higher levels of these promising functional ingredients.

Keywords: Processed cheese spread, Functional ingredient, Bee pollen, Palm pollen, Wheat germ, Amino acids, Sensory evaluation.

Introduction

Recently, the interest in healthy and functional foods with high nutritional value has increased, and consumers look for foods with natural ingredients that have a good effect on health and lifestyle habits. Processed cheese is one of the most popular cheeses which is considered a homogenous emulsions of different dairy and non-dairy ingredientsin the presence of emulsifying salts and heat (Ferrão et al., 2016). It differs from traditional cheeses since it is obtained by some sequential technological processes and whose properties depend on the ingredients included in their formulations and it is present in different forms in market (Nastaj et al., 2020). As a food with high nutritive value containing different forms of nutrients such as proteins, fats, carbohydrates, minerals and vitamins, processed cheese can be included in diets providing some beneficial health claims when combined with natural functional and bioactive ingredients (Solhi et al., 2020; Vásquez et al., 2018). Different research approaches have been implemented to fortify the processed cheese with some bioactive and functional ingredients (herbs and spices extracts, essential oils and vegetables) to enhance its physical, chemical, microbiological and functional properties (Solhi et al., 2020). In addition, the quality properties of processed cheese highly depend on its texture, rheology and composition beside the manufacturing circumstances (Solhi et al.,

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2020). Bee pollen, the highly nutritive food, is composed of around 45-60% carbohydrates, 15-25% proteins, 5-10% fats and 10-19% fibers beside its valuable content of minerals (manganese, zinc, potassium, magnesium, selenium) and vitamins. The daily intake of 50 gram of bee pollen can provide the human with most essential vitamins and minerals covering up to 50% of the recommended daily intake (Thakur et al., 2020). Further, consuming 15 gram of bee pollen can provide the human body with its amino acid requirements as well as decreasing the cholesterol level (Thakur et al., 2020). The easy way for increasing its consumption is to incorporate bee pollen into routinely consumed foods. Moreover, it contains amino acids, high phenolic and flavonoid content with antioxidant, antimicrobial, anti-diabetic, anticancerand anti-inflammatory activities(Rasouli et al., 2018) and it is considered as "the best food product in the world" and is an excellent functional ingredient (Kieliszek et al., 2018; Sokmen et al., 2022). It was used in different food application including bakery, meats, confectioneryand juice (de Florio Almeida et al., 2017; Turhan et al., 2017; Conte et al., 2018).

Additionally, wheat germ is another functional food with high nutrition and health benefits and plays a vital role in human nutrition. It consists of carbohydrates (18-24% starch), proteins (21.5-28.3%) fats (8-11%), fibers, minerals (magnesium, zinc, potassium, phosphorus) and vitamins E and B group. Also, wheat germ has some biologically active compounds such as phenols, flavonoids, steroids, glutathione, unsaturated fatty acids and some essential amino acids beside its technological and functional activity for absorbing water (Nahla et al., 2018; Tekgül et al., 2021). It could be a promising functional ingredient which can be involved in our diet.

In addition, palm pollen is another natural dietary food supplement produced from the male palm flowers and consists of 1-13% carbohydrates, 1% fiber, 1-20% fats, 15-31% protein, 4-7% ash and 1.07% reducing sugars (Sebii et al. 2019). It also contain fatty acids, amino acids, vitamins E, A and C and minerals such as Se, Zn, B, Mn, Mo, Ni, Cu and Fe. It could be one of the health promoting factors due to its content of polyphenols, flavonoids and antioxidantswhich motivate toward the production of newly functional products (Echegaray et al., 2021; El-Kholy et al., 2019). Therefore, this work aimed to supplement processed cheese with natural and functional ingredients (bee pollen, palm pollen, wheat germ) and to study its effects on some physicochemical, functionality, amino acid components and sensorial properties of processed cheese.

Materials and Methods

Ingredients

Different cheeses (Ras, Cheddar), skim milk, butter and non-dairy ingredients (bee pollen, palm pollen, wheat germ) were purchased from local marker, while tri-sodium citrate (Sigma-Aldrich, Germany) was used for emulsification. The chemical composition of raw materials used in processed cheese formulations is presented in Table 1.

Experimental procedures of processed cheese

All experimental processed cheese treatments were conducted in Dairy Department Laboratory, Faculty of Agriculture, Fayoum University as follows:

Use a mixture of cheddar cheese, Ras cheese and butter in the manufacture of processed cheese in a combination consisting of(38.40% Ras cheese, 12.80% cheddar cheese, 10.30% butter, 3.00% emulsifying salts, and 35.50% water) according to Rafiq et al. (2017) with some modifications. Cheeses were milled by blender and placed with butter into double jacketed tank. Then, it is dissolved3.00% tri-sodium citrate in the appropriate amount of warm water andit is added three times during heating the basic cheese. Cooking is carried outat 85–95°C using direct steam and various individual additives(bee pollen, palm pollen, wheat germ)were added to homogeneous basic cheeseat level 5% in their examined treatments, while skim milk powder was used in control processed cheesed at level5%. The mixture was cooked at mentioned temperature for 5–10 min with continuous stirring,it poured into containers of known size, cooled for two hours at room temperature, and kept in the refrigerator until analysis.

Methods of analysis

Chemical analysis

Fat, moisture, ash, carbohydrateand protein contents were determined as described in(AOAC, 2005). The pH values of processed cheese treatments were measured using laboratory pH meter with a glass electrode Model pH (Kent EIL 7020).

TABLE 1. Chemical composition of raw materials used in the preparation of processed cheese .

Material	(%) Chemical composition					
	Protein	Fat	Carbohydrate	Moisture	Fiber	Ash
Ras cheese	22.16	25.65	1.29	45.47	--	5.52
Cheddar cheese	25.36	33.70	1.51	34.16	--	5.27
Butter	--	82.00	--	16.00	--	0.5
Skim milk Powder	34	1.5	52	4	--	8.5
Bee Pollen	32.8	12.8	40.7	3.45	8.65	1.6
Palm Pollen	38.06	19.57	21.56	9.85	3.22	7.74
wheat germ	34.51	10.4	28.3	8.69	13.6	4.5

Physical properties

Meltability and oil separation of resulting processed cheese samples were measured using a modified test according to the described methodbyAbbas et al. (2021).

Free amino acids

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, and Cairo, Egypt. The GC was equipped with DB-WAX column (30 m x 250 µm internal diameter and 0.25 µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.90 mL/min at a split less, injection volume of 1 µLand the following temperature program: 50°C for 1 min; rising at 25°C /min to 200°C and held for 5 min; rising at 3°C/min to 220 °C and held for 10 min; rising at 5°C/min to 240°C and held for 8 min. The injector and detector were held at 250 °C and 290°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 60-400 and solvent delay 6 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data (Abdalla et al., 2022).

Total phenols and antioxidant activity

The Folin-Ciocalteau assay was used for the determination of total phenols and the results were expressed as mg GAE/g according to Guimarães et al. (2020), while the antioxidant activity was determined using DPPH method as percentage of inhibition (Ali et al., 2019).

Sensory evaluation

Processed cheese samples were sensory evaluated using a nine point hedonic scale for determining the following parameters; appearance, color, flavor, creaminess, firmness, spreadability, overall impression as described by Fan et al. (2023).

Statistical analysis

All analyses were carried out in triplicate and the results reported as means± standard deviation. The data obtained were statistically analyzed by general linear model using SPSS software (2007) with Duncan›s multiple range tests at $p \leq 0.05$.

Results and Discussion

Chemical composition of spreadable processed cheese

Table 2 indicated the main values of moisture, fat, protein, ash, carbohydrates, Fat/DM and pH ofspreadable processed cheese produced using Bee pollen (BP), Palm pollen (PP) and wheat germ (WG). The incorporation of BP, PP and WG into processed cheese increased the total solids of the product compared to control processed cheese. While, the moisture content of different processed cheese samples decreased,this isdue to the hightotal solids of such additives. At fresh time, it was observed that the highest moisture content (51.23%) was recorded in control processed cheese followed by processed cheese with PP-cheese (49.47%) and BP-cheese (49.34%). The lowest moisture content (48.29%) was recorded in WG-cheese with a significant difference ($P \leq 0.05$) in comparison to other processed cheese samples. This might be due tothe reduction of retained water in cheese matrix resulting about the interactions between milk components and polyphenols of different additives as reported by Abd Elhamid et al. (2017). Moreover, wheat germ has a higher content of carbohydrates, fibers and proteins which interact and reduce the free water

in cheese matrix (Majzoobi et al., 2016). The lower moisture in all processed cheese samples compared to control might be due to the high dry matter in such additives and the relatively high concentrations of proteins, ash and carbohydrates of BP, PP and WG. The increase in fat, proteins, carbohydrates and ash during storage time was due to the moisturelost from the processed cheese samples. In addition, at fresh time, was observed the high contents offat and carbohydrate in WG-cheese while the lower content was noted inthe PP-cheese and control cheese. Moreover, the protein and ash contents were higher in PP-cheesecompared to other cheese samples. Ash content of BB, PP and WG-cheese was significantly different ($P \leq 0.05$) compared to control cheese at fresh time of storage. This might be due to these additives containing reasonable amounts of ash which affected theash content of produced cheese. The highcontent ofFat/dry matter was determined in control processed cheese when compared with other cheese samples.

There were no significant differences ($P \leq 0.05$) in the pH values among all processed cheese samples. Different pH values between samples were obtainedas a result of increased acidity of cheese sampleswhich might be due to the enhancement of microbial activity by such additives;this is consistent withKostić et al. (2020). It wasfound that wheat germ has a valuable content of amino acids, fatty acids and antioxidants with acidic nature which reduces the pH of processed cheese as reported by Majzoobi et al. (2016). These results were in the same linewith Atallah (2016) who used Bee pollen grains in the production of bio-yoghurt and there was an increase in in total solids, fats, proteins, ash and carbohydrates of the product. In addition toAbbas et al. (2023),who indicated the same when used Bee pollen in the production of soft cheese. Nahla et al. (2018)Indicated that the higher contents of total solid in soft cheese which is due to the use of wheat germ in its production, which leads to an increase in fat, proteins and ash?Jamdar et al. (2021) and Çetinkaya et al. (2020) also found that using wheat germ in the production of white cheese led to the increase of fats, proteins, ash and acidity of cheese. In addition, Seleet et al. (2016) indicated the same pattern when used wheat germ in the preparation of fermented dairy product. Moreover, Bee pollen isconsidered a good source of carbohydrates in a digestible state and contains glucose and fructose as reducing sugars (El Ghouizi et al., 2023).

Physical properties of spreadable processed cheese

The physical properties of spreadable processed cheese with BP, PP and WG were shown in Table 3. It could be noticed that addition of BP and PP decreased the meltability and oil separation of processed cheese while WG increased meltability and oil separation of processed cheese. The highest meltability and oil separation values were observed in WG-cheese while the lowest ones were obtained in PP-cheese. These findings were in line with Khalifa et al. (2020), who observed the same pattern in spreadable processed cheese produced with quinoa flour, and with (Hamdy et al., 2021) who used oat flour in the production of spreadable processed cheese. Meltability and oil separation of spreadable proceeded cheese increased in all samples during storage,this is consistent with Tawfek (2018) and Hamdy et al. (2021). This gradual increase during storage might be due to the interactions between proteins and emulsifying salts as well asthe higher dissociation of casein (Hamdy et al., 2021). Increase in physical properties valuesof processed cheese during storage time might also due to the cracking occurred in processed cheese matrix and the higher level of protein degradation (Hamdy et al., 2021). The low level of meltability in PP-cheese was due to its lower content of dietary fiber compared to BP and WG-cheese. On the other hand, the high level of meltability in WG-cheese was due to its higher content of dietary fibers which affect the textural properties of cheese matrix (Alqahtani et al., 2023). The high protein content in BP, PP and WG is affected bythe physical parameters of produced processed cheese which is due tothe interactions betweenprotein-protein and protein-fat (Abdeen et al., 2018). In addition to, the interaction between wheat germ proteins, fats, carbohydrates and fiber as well as dairy proteinswhich affectthe physical properties of processed cheese (Majzoobi et al., 2016). Bee pollen has been shown to have emulsifying propertiesand high solubility of carbohydrates which affects physical properties of processed cheese(Thakuret al., 2020).

Total phenols and antioxidant activity

Figures **1** and **2** shows the phenolic contents and antioxidant activity of processed cheese treated with BP, PP and WG. The incorporation of different additives increased the level of phenols and antioxidants of processed cheese samples. High levels of phenolsand antioxidant activity were obtained in PP-cheese when compared to other processed cheese samples, while the

low ones were obtained in control cheeses. This is due to the available content of phenolic flavonoids and antioxidantsforsuch additives. These results are consistent withAlqahtani et al. (2021) and Hamdy et al. (2021). Palm pollen was considered as an effective natural ,functional food ingredient and a good source of bioactive components such as phenols and flavonoids possessing higher antioxidant activities which can be usedas a health promoter in food system preparations (El-Kholyet al., 2019). In addition, Echegarayet al. (2021)it has explained that palm pollen contains considerable content of phenols and flavonoids which make it an important source of such bioactive compounds. Also, Bee pollen has a valuable content of phenolic compounds such as flavonoids, phenolic acids, carotenoids and anthocyanins with apparent antioxidant activity (Kaškonienė et al., 2020). There are about1.6% phenolic compounds represented in phenolic acids (0.2%), flavonoids (1.4%) and catechins(El Ghouizi et al., 2023).The presence of such bioactive componentsin Bee pollen, it has a significant antioxidant, anti-carcinogenic and hepatoprotective activities beside its cardioprotective action(Kostić et al., 2020). Therefore, Bee pollen is considered as a natural functional ingredient used in the formulation of functional foods with health promising and health benefits.Moreover, wheat germ increased the phenolic content and antioxidant activity of processed cheese due to its content of phenolic compounds with antioxidant actions. These results were in accordance to Nahla et al. (2018), who used wheat germ in the production of soft cheese and they showed a clear increase inthe phenolic content of cheese and El-Kholy et al. (2019), who used date palm pollen in the manufacture of yoghurt with higher phenolic and antioxidant activity. Karabagias et al. (2018) showed improvement in antioxidant activity and the phenolic content of yoghurt with the addition of bee pollen and Thakur et al. (2019) Developed polyphenol-rich milk powderwith the addition of bee pollen. Darwish et al. (2020)also decided the same resultsin the manufacture of processed cheese with the addition of date seed powder. Majzoobi et al. (2016) foundhigher content of phenolic compounds and antioxidant activity indairy dessert fortified with wheat germ. This might encourage researchers to producenew functional products with the inclusion of these natural ingredients to fulfill the consumer demands.

Amino acid profile

Table 4 demonstrates the amino acid composition of processed cheesewith different additives BP, PP and WG. The incorporation of such additives in processed cheese enhanced the amino acid concentration.Sixteen amino acids were detected in processed cheese samples. In addition,eight essential amino acids(Histidine, Threonine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, and Lysine) and eight non-essential amino acids were identified (Proline, Aspartic, Glutamic, Serine, Glycine, Arginine, Alanine and Tyrosine). A balanced amino acid profile was observed in all processed cheese samples with different additives.The essential amino acids (Methionine, Leucine and Phenylalanine) were detected in higher concentrations in comparison to other amino acids. BP-cheese had the highest level of the three aforementioned acids, while WG-cheese had the lowest ones. Bee pollen had the superior effect on the amino acid concentration followed by palm pollen and wheat germ respectively. The essential amino acids were present in all experimental processed cheese with comparable values.

Bee pollen, Palm pollen and Wheat germ were considers as good sources of protein and essential amino acids. About 10% of Bee pollen protein is essential amino acids including leucine, threonine, methionine, isoleucine, histidine, lysine, phenylalanine, tryptophan and valine (El Ghouizi et al., 2023). In addition, wheat germ has range of essential amino acids forming protein in high quality (Ghelich et al., 2022). These results consistent withGhelich et al. (2022), who found higher content of amino acids in yoghurt supplemented with wheat germ.Seleet et al. (2016) also found higher content of amino acids in the dairy fermented product supplemented with wheat germ. Moreover, Sebii etal (2019) and Echegaray et al. (2021) reported that palm pollen had all essential amino acids which might increase its functionalproperties , which promote the results of this studyfor improving theamino acid profile of spreadable processed cheese.

Sensory characteristics

Sensory evaluation of processed cheese samples with different additives BP, PP and WG is shown in Table 5. Appearance was significantly ($P \leq 0.05$) affected by the addition of such additives and the highest score were recorded in control processed cheese, while the lowest one was observed in PP-cheese. During storage, some processed cheese samples were evaluated with comparable appearance values as control cheese such as WG-cheese. The values of color was not significantly ($P \leq 0.05$) different between all processed cheese samples which means that panelists accepted the color of produced processed cheese. Although, Çalışkan Koç et al. (2019) reported that WG increased the darkness