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Nutritional Quality and Sensory Attributes of Biscuits Fortified with Bee Pollen

Fatma M. Abd-Elmegaly; Souzan S. Latif; Sanaa, A.M. Saleh and Sanaa M. Abdel-Hameed
Food Science Dept., Fac. of Agric., Minia Univ., Minia, Egypt.

Abstract - The objective of this study was to investigate using bee pollen (BP) 2.5, 5, 7.5, and 10% as a functional ingredient in biscuits. At zero time and after eighteen days, the physicochemical and sensory properties of enriched biscuits were evaluated in terms of total phenolic content (TPC), total flavonoid content (TFC), and β-carotene. The addition of pollen decreased the L value with increasing in biscuits. The enriched BP10% biscuit had a TPC content of 21.15±0.10 mg/100 g, 182.38±1.92 mg/100 g as TFC, and 9.73±0.11 mg/100 g as β-carotene. Furthermore, the biscuits made with the inclusion of BP were accepted in terms of their sensory qualities. The highest hardness and crispness loss occurred during the first twenty days of storage. There were no pathogenic bacteria or fungal growths in our products. Depending on the obtained data, it is possible to conclude that bee pollen is a component that can increase quality standards while also boosting the functional elements of biscuits.

Keyword: physicochemical, color characteristics, total phenolic, β-carotene, sensory qualities

Introduction

Biscuits are a popular ready-to-eat bakery food among consumers of all ages due to their good flavor, variety, stability, and convenience. Because of their health benefits and greater quality, various types of biscuit fortified with phenolic acids have gained popularity in recent years [1]. Biscuits are popular not only because of their sensory profile and simplicity of eating, but also because of their ease of storage and long shelf life. Commercial biscuits have a low protein level of approximately 7 to 10% [2]. Since of their low protein content, protein enrichment of biscuits and other cereal goods is appealing because it improves nutritional qualities and enhances protein consumption.

As a result, considerable effort has been expended on inventing food products that can improve people's health. While overweight and obesity are increasing across the board in developing nations, undernutrition persists and goes hand in hand with weight gain and the burden of dietary-related illness. Furthermore, recent outbreaks of viral diseases such as malaria, Ebola virus disease, the HIV pandemic, and the COVID-19 crisis emphasize the importance of eating a well-balanced diet [3].

Honey bees (*Apis Mellifera*) collect pollen from plants and store it on their hind legs, enriching it with salivary enzymes to produce microscopic granular grains known as "bee pollen." Bee pollen is often referred to as "apiculture pollen," "corbicular pollen," or "bee gathered pollen" [4]. Due to these properties, bee pollen is regarded as one of the oldest nutritional supplements in history, including nearly all of the nutritious components for a diet. In recent years, it has been described as "only totally complete

Corresponding author E-mail: mmrmr7977@gmail.com

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food" and the world's best food product [5,6]. These dietary profiles meet the colony's primary sources of carbohydrate and protein nutrition, with protein content ranging from 10% to 40%, carbs ranging from 13% to 55%, and lipids ranging from 1% to 10%. [7]. Many studies have found that pollen contains 15-19 amino acids, including all necessary amino acids. Pollen has numerous medicinal qualities such as antimicrobial, antitumoral, antibacterial, immunomodulator, anti-inflammatory, and nutritive properties since it is rich in phytochemicals such as phenolic acids, flavonoids, and carotenoids [8-10]. Although of these circumstances, the practical application of pollen in food processing remains limited [11-12]. The addition of pollen to a food matrix often improves the nutritional, functional, technological, and sensory qualities of newly formulated food products due to the concentration. This potential for improvement is determined by pollen's physical, functional, thermal, and textural qualities [11,13]. Our aim was producing functional biscuit including pollen and expand the application as a potential alternative.

Material and methods

Materials:

For this study we used half kilogram of pollen was purchased from the South Valley University. All chemicals used in this study were of analytical grade and purchased from El-Naser pharmaceutical chemicals.

Methods:

Preparation of pollen

Bee pollen was collected from beehives located in the South Valley University This pollen was dried at a temperature of 40 °C to achieved moisture level of 10 %. Then it was ground and sieved to obtain its powder form.

Preparation of biscuits:

The supplemented biscuits were prepared using the formula in table (1) according to R.sai Manohar and P. Haridas Rao 1996.Wheat flour (WF) was replaced with bee pollen (BP) at 2.5, 5, 7.5 and 10% levels. The processing steps of biscuit preparation are illustrated in Fig (1). Samples were packed in metalized Orientated Polypropylene (OPP)/paper multilayer bags in air, and a filled weight of 200 g.

Chemical analysis:

Moisture, crude protein, crude fat, ash and crude fiber contents were determined according to the methods of the [12]. Nitrogen free extract (NFE) was calculated by difference. All determinations were performed in triplicates and the means were reported. Energy values were calculated as reported by [14] applying the factors, 4, 9 and 4 for each gram of protein, lipids and carbohydrate, respectively.

Determination of total phenols:

Estimation of total phenols was carried out according to [15] using Folin-Ciocalteu reagent. Approximately 10 g sample was homogenized with 100 mL extracting solvent (methanol 50%) for 1 min under high speed. The extracted samples were centrifuged for 15 min at 3000 rpm. The supernatants were collected and passed through Whatman No.1 filter paper. About 0.50 mL sample extract was added with 2 mL distilled water and 2.50 mL diluted Folin-Ciocalteu reagent

(0.20 N). The samples (extracts with Folin-Ciocalteu reagent) were left for 5 min before 5 mL of 7.5% (w/v) Na_2CO_3 was added. The absorbances were taken at 765 nm after 2 hrs. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The results were expressed as mg of gallic acid equivalents/100g of sample.

Determination of total flavonoids compound:

Aluminum chloride colorimetric method was used for flavonoids determination according to [16]. One milliliter of sample extract (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10 % aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. the absorption of the reaction mixture was measured at 510 nm. The concentration of the flavonoids was expressed in terms of mg quercetin of /100 g. All samples were analyzed in triplicate.

Total carotenoids:

The procedure involved extracting with acetone as follows: samples were mixed with 50 ml acetone for 20 min in the dark, and the mixture was filtered through carded cotton [17]. Carotenoids were obtained by using the acetone method described above. Finally, all samples were measured the absorbance at 450 nm.

Determination of color:

The color characteristics of samples were measured by a color difference meter (model color Tec-PCM, USA) using different color parameters (L, a, b) according to [18]. In addition, numerical total color difference [19].

Microbiological analysis:

The total Microflora count was determined according to [20]. using nutrient agar media

Sensory evaluations:

Sensory evaluation for the color, flavor texture and overall quality were done in order to determine consumer acceptability. A numerical hedonic scale which ranged from 1 to 10 (1 is very bad and 10 for excellent) was used for sensory evaluation [21].

Statistical analysis:

The statistical analysis was carried out according to (SPSS) software program. All results are presented as arithmetic means ± SD. Means and standard deviation (SD) were measured and Duncan's multiple range test at 5% significant level.

Results and discussion

Chemical composition of wheat flour (WF) and bee pollen (BP):

The approximate chemical composition of wheat flour (WF) and bee pollen (BP) are shown in Table (2). Results showed that the bee pollen contained 87.5±0.18% of dry matter, 43±0.34% of protein, 6.40±0.25% of fat, 2.66±0.12% of ash, 1.42±0.02% of fiber, 46.5±0.09 of NFE and 48±4(mg/100g) of vitamin C. Wheat flour had less ash (0.62±0.03%) and protein (9.44±0.03%), but more fibers (3.32±0.02%) than bee pollen. In light of this, bee pollen was proven to be a good supplier source of protein and ash. As a result, it could be used it as a nutritious ingredient in the production of healthy food products. When calculated and reported as Kcal/100g sample, the energy values for BP and WF in the same table were 382.02 and 396.94, respectively. Additionally, there was a high agreement between the data in Table (1) and those published by [22-24].

Total polyphenol, Flavonoids and β-carotene contents in fortified biscuits:

Bee pollen contains a high concentration of phenolic components such as cinnamic acid derivatives, flavonoids, flavones, isoflavones, anthocyanins, and flavonols [25-28]. Enriching biscuits with bee pollen, which is high in these chemicals, should have a considerable impact on their content in the finished products [29]. The total phenolic content of biscuits containing 2.5 to 10% pollen increased in comparison to the control, when expressed as gallic acid (Table3). Based on the findings, it can be concluded that the level of bee pollen addition rose in line with the total amount of total polyphenols in biscuits, as well as their portion.

In summary, the results demonstrate that a 10% addition of pollen is the most beneficial in terms of enriching biscuits in phenolic compounds (Table 3). The total phenol was three times higher at 10% bee pollen addition compared to the control sample, indicating that this level of supplementation appears to be ideal for providing maximum nutritional advantages. Approximately 10-50% of the phenol content of the samples were lost throughout storage.

Flavonoids are predominantly metabolized in the small intestine by enzymes derived from gut microflora, which breakdown the flavonoids to phenolic acids [30]. Phenolic acids are quickly digested or absorbed in the liver. Our results show that a 10% pollen addition is the most efficient in terms of enriching biscuits in flavonoid compounds (Table 3). Flavonoids were more than 40%, 24%, and 15% increased with 10%, 7.5%, and 5% pollen addition compared to the control sample, respectively. After 80 days, BP3 and BP4 significant lost about 6-16% of their flavonoid content.

Generally, total phenolic content (TPC) and total flavonoid content (TFC) of bee pollen from Portugal, the United States, Brazil, China, Egypt, New Zealand, and Greece ranged from 0.50 to 213 mg GAE/g and 1.00-5.50 mg QE/g, respectively [31-32]. This could be due to differences in meteorological circumstances and geographical origin, which alter the quantities of phenolic chemicals in plants [33].

Vitamin A in the form of β-carotene has been found in bee pollen from Thailand (1.530 mg/100 g) and Southeastern Brazil (5.63-19.89 mg/100 g) [34]. Our samples were contained more β-carotene than the control sample; BP4 was considered more than double. During the storage period, β-carotene levels in all samples did not change significantly. This seems significant to get the manufacturers' attention so they can include pollen-fortified biscuits to their product range because they are a very potential source of nutrition.

Color

Color influences the selection and consumption of food products, especially biscuits [35-36]. The addition of bee pollen significantly darkened the surface of the biscuits as compared to the control, as shown by Table 4 measurement of the color of the biscuits. In contrast to the control sample, the values of the L* parameter stayed at a lower level. This is of course, expected given that bee pollen is noticeably darker in color than wheat flour. Furthermore, because color develops during the later stages of baking [37].

During baking, macro-molecule compounds known as melanoidins were generated as a result of this non-enzymatic process. Thus, the color of biscuits develops as the temperature and baking time increase [38], and is affected by the number of sugars and proteins contained in the baking components. Because bee pollen had more proteins than wheat flour and the reducing sugars in the dough came from pollen, the amount of melanoidins generated in enriched cookies was much higher than those in the control.

Food color changes during storage also convey information about its freshness, and thus its shelf life [39]. However, no modifications were found as a result of biscuit storage, regardless of the amount of the ingredient. The variations in the a* parameters were often not statistically significant, however the difference in the b* parameter between zero-time samples and those held for forty and eighty days was statistically significant (Table 4).

Changes in moisture content of biscuit samples during storage

The increase in moisture during storage altered biscuit texture features such as hardness and crispness loss (Fig. 3). The highest hardness and crispness loss occurred during the first Twenty days of storage, in correspondence with the fastest increase in water content in the biscuit matrix, and followed a similar trend throughout the storage duration. The frequency of texture changes diminished in the remaining part of the storage; during this period, the biscuits had the largest moisture content, and as a result, they probably underwent plastic deformation, and a likely redistribution of the fat inside the product matrix happened [40].

As previously observed [41], water absorption by the dry matrix and an overall rise in moisture content cause swelling of the food polymers, exposing sorption sites with higher binding energies and resulting in a decrease in material porosity. Water uptake in the matrix, even if minor, could have behaved as a plasticizer, decreasing crispness and hardness while enhancing flowability and softness and favoring viscous behavior [42]. Moisture content is recognized as key factors influencing hardness in dry food and biscuits [42-43]; however, texture is also influenced by other physico-chemical aspects such as product composition (e.g., interactions between ingredients such as fat, sugar, starch) and its micro- and macro-structure characteristics. According to [40] the change in the structure of biscuits is largely reliant on the amount of fat in the product formulation, and hence on the material qualities.

Total Microbial Load

A total count of microorganisms was performed on the biscuit samples after manufacturing and during storage at room temperature. The results were consistent with Egyptian Standard Specifications for Biscuits No. 5117 for 2006. There found no pathogenic bacteria or fungal growths in our products.

Sensory characteristics of biscuits

There were major variations between the control and the pollen-enriched biscuits. Fig.4 represents the sensory evaluation. According to the results, bee pollen-enriched biscuits received a higher overall score. Fortification at a level of 5% and 7.5% retained the sensory metrics better than the control sample in both fresh and stored biscuits. It should also be highlighted that at all pollen concentrations, the overall assessment permitted the product to be classified as acceptable (total score 7.79) in the case of the maximum additive BP2 sample). Biscuits 2.5%.and 10% received lower scores when substitution was set at 5% and 7.5%, respectively (figure 4).

When comparing individual sensory qualities of biscuits immediately after baking and after storage, significant variations were found between the control and the sample with the 5 and7.5% bee pollen additions (figure 4). After storage, there were statistically significant differences. On this basis, it is possible to conclude that the 5% bee pollen fortification of biscuits was feasible and yielded the intended outcomes.

Taste, consistency, and fracture have all deteriorated in particular. When used in high quantities, bee pollen was a key contributor to the loss of biscuit flavour; when used in big quantities, the biscuits left a subtle bitter aftertaste in the mouth. Further, in addition to the previously mentioned characteristics, the color of the product is affected by the chemical compositions of bee pollen, which is influenced by plant species and environmental conditions [44]. As a result, the flavonoids and carotenoids in the product, as well as the reducing sugars supplied with bee pollen, had an effect on its color.

Conclusion

In accordance with the findings of this study, honeybee pollen is a good source of healthful bioactive natural compounds such as polyphenols, flavonoids, and β-carotene. They can also be used as functional foods for children and Adolescent.

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Table (1): Formula of fortified biscuit with pollen Control (100% WF); BP1 (2.5%); BP2 (5%); BP3 (7.5%) and BP4(10 %), Parameters in columns denoted with the same letters do not differ statistically at the level of confidence α = 0.05. Number of replications n = 3.

ingredients (g)	Control	BP1	BP2	BP3	BP4
Wheat flour	100	97.5	95	92.5	90
Butter	20	20	20	20	20
Sugar	30	30	30	30	30
Eggs	10	10	10	10	10
Baking powder	0.3	0.3	0.3	0.3	0.3
Bee pollen	0	2.5	5	7.5	10

Table (2) Chemical properties of bee pollen and Wheat flour

Constituents (%)*	Bee pollen(BP)	Wheat flour(WF)
Dry matter	87.5±0.18	88.55±0.16
Ash	2.66±0.12	0.62±0.03
Protein	43±0.34	9.44±0.03
fibers	1.42±0.02	3.32±0.02
Crude fat	6.40±0.25	2.54±0.06
NFE**	46.52±0.09	84.08±0.70
Vitamin C (mg/100g)	48±4	ND
Energy value (Kcal/100g)	382.02	396.94

Table (3) The effect of storage bee pollen-fortified biscuits at room temperature (205°C) on the total phenolic, flavonoid, and β-carotene contents

L value					
	Zero time	**20 days**	**40 days**	**60 days**	**80 days**
Control	69.46±0.11^{a}	68.39±0.57^{b}	68.50±0.01^{c}	68.93±0.11^{c}	63.33± 0.57^{d}
BP1	63.53±0.11^{d}	63.50±0.001^{d}	63.0±1.03^{e}	61.90±0.01^{f}	60.83±0.11^{g}
BP2	62.33±0.30^{g}	61.80± 0.2^{g}	60.86±0.11^{H}	60.53±0.11^{I}	59.2± 1.03^{I}
BP3	61.73±0.11^{I}	61.4± 0.34^{IJ}	60.8±0.11^{IJ}	60.60±0.2^{IJ}	60.53±0.11^{IJ}
BP4	59.83±0.11^{J}	59.76±0.11^{K}	60.03±0.11^{K}	60.46±0.11^{K}	60.56±0.23^{L}
a value					
	Zero time	**20 days**	**40 days**	**60 days**	**80 days**
Control	3.76±0.23^{a}	3.46±0.11^{b}	3.30±0.01^{c}	3.40± 0.2^{c}	3.40± 0.2^{d}
BP1	4.36±0.23^{d}	3.80±0.01^{de}	3.66± 0.11^{ef}	3.53±0.11^{ef}	3.36± 0.23^{fg}
BP2	7.53±0.11^{fg}	7.16±0.05^{gH}	7.33 ±0.57^{H}	6.86±0.11^{H}	6.76± 0.23^{I}
BP3	8.00±0.01^{J}	7.63±0.11^{JK}	7.00±0.01^{JKL}	7.16±0.57^{KIM}	3.43±0.34^{LM}
BP4	9.13 ± 0.46^{LM}	8.83±0.11^{LM}	7.93±0.11^{LM}	7.60± 0.34^{M}	7.33±0.57^{M}
b value					
	Zero time	**20 days**	**40 days**	**60 days**	**80 days**
Control	32.66± 0.30^{a}	32.36±0.32^{ab}	31.66±0.41^{b}	31.56± 0.11^{c}	31.16±0.57^{c}
BP1	29.43± 0.23^{c}	29.36± 0.23^{d}	30.00±0.01^{e}	30.16±0.05^{e}	30.32±0.05^{e}
BP2	30.82±0.29^{e}	30.86±0.11^{f}	29.93±0.11^{f}	29.73±0.41^{fg}	28.93±0.11^{g}
BP3	31.83±0.11^{g}	31.63± 0.23^{g}	31.16±0.57^{H}	30.93±0.11^{HI}	30.33 ± 0.57^{IJ}
BP4	30.33 ± 0.57^{IJ}	33.53±0.11^{J}	33.70± 0.4^{K}	32.70± 0.4^{K}	32.70± 0.4^{L}

Table (4) The colour parameter of bee pollen-fortified biscuits storage at room temperature (20-5 °C).

Total phenol			
	Zero time	**40 days**	**80 days**
Control	8.85± 0.03[a]	8.69±0.09[b]	8.51±0.17[c]
BP1	11.64± 0.08[d]	10.44±0.34[e]	10.02±0.03[f]
BP2	15.73±0.01[g]	12.75±0.13[H]	11.01± 0.18[I]
BP3	18.78± 0.01[J]	14.48±0.73[K]	13.24± 0.25[L]
BP4	21.15±0.10[M]	18.39±0.39[N]	13.77± 0.19[O]
Total flavonoid			
	Zero time	**40 days**	**80 days**
Control	130.16±0.01[a]	128.50±3.33[b]	142.38±3.84[c]
BP1	137.38±1.92[d]	133.50±3.33[e]	135.16±3.33[f]
BP2	150.72 ±1.92[g]	160.72±2.75[H]	137.94±10.71[I]
BP3	161.27± 1.92[J]	154.05±1.92[K]	151.83±6.66[L]
BP4	182.38±1.92[M]	169.61±1.92[N]	165.16±8.81[O]
β-carotenes			
	Zero time	**40 days**	**80 days**
Control	5.50±0.01[a]	5.33±0.11[a]	5.33±0.11[a]
BP1	7.10± 0.34[b]	6.93± 0.11[bc]	6.93± 0.11[bc]
BP2	7.93±0.11[bc]	8.10± 1.21[c]	8.10± 1.21[c]
BP3	8.26± 0.50[d]	7.90±0.01[e]	7.90±0.01[e]
BP4	9.73±0.11[f]	9.66±0.11[f]	9.66±0.11[f]

Control (100% WF); BP1 (2.5%); BP2 (5%); BP3 (7.5%) and BP4(10 %), Parameters in columns denoted with the same letters do not differ statistically at the level of confidence α = 0.05. Number of replications n = 3.

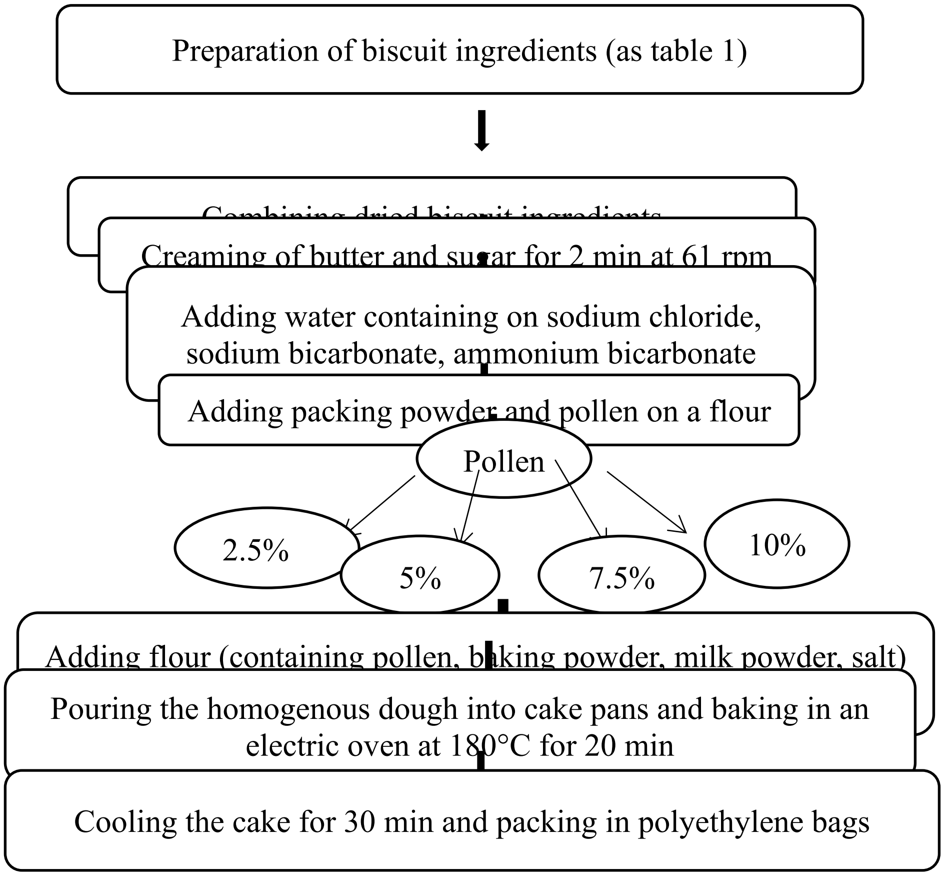

Fig. (1): The processing steps of fortified biscuit with pollen

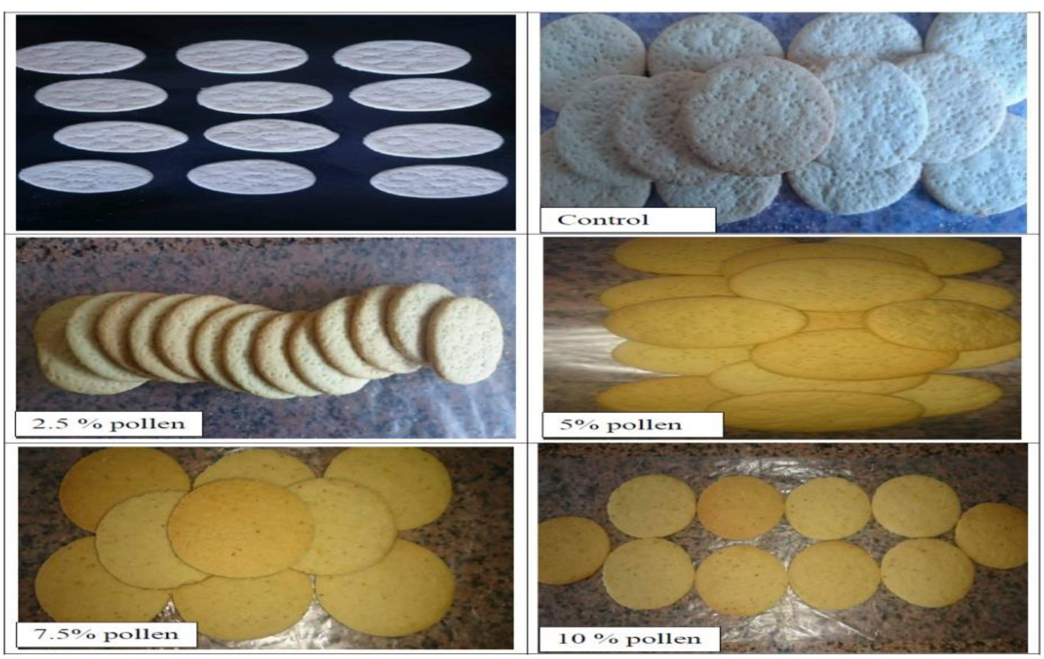

Fig. (2) Fortified biscuit with bee pollen

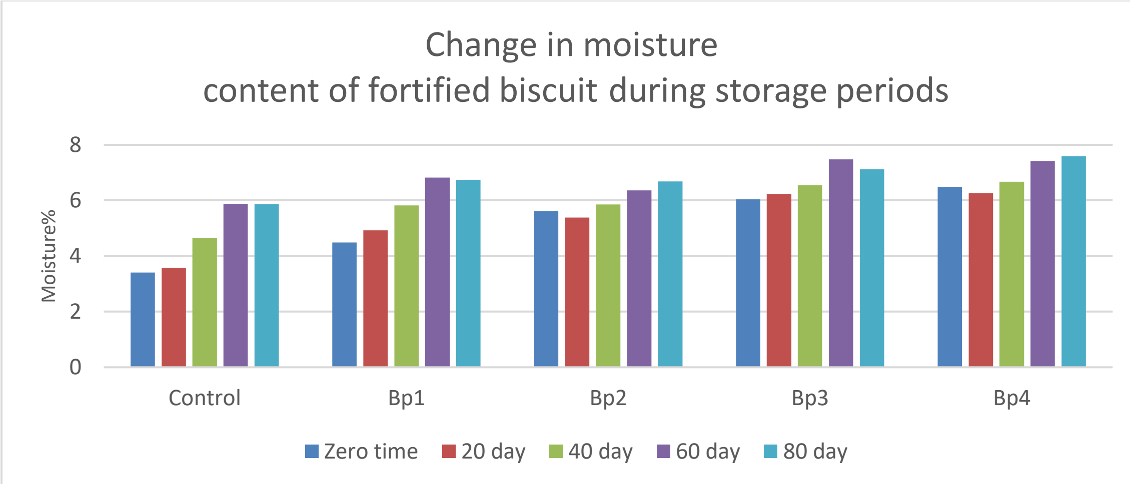

Fig. 3. Changes in moisture content of biscuit samples during 80 days of storage at room temperature (20±5°C)

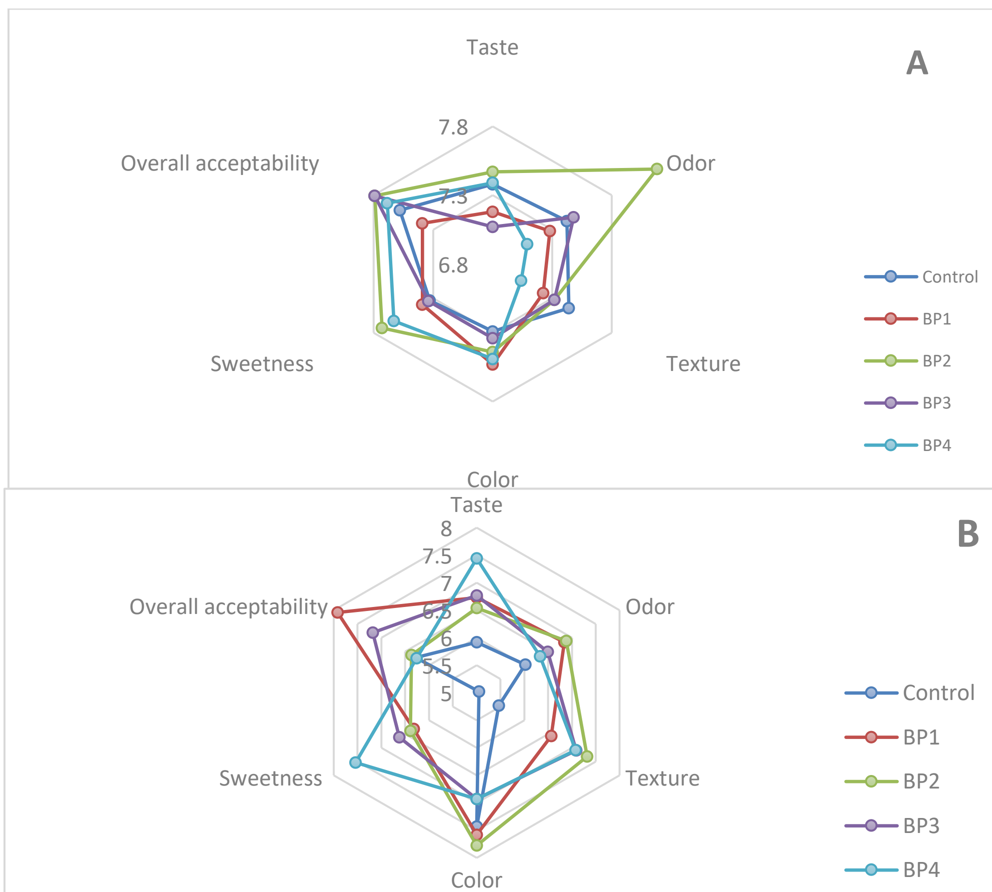

Fig. 3. Changes in Sensory characteristics of biscuit samples at zero time (a) and 80 days (b) of storage at room temperature (20±5°C)