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Influence of Bee Pollen on the Bioactive Behavior, Sensory and Physicochemical Properties of White Cheese Made from Camel and Cow Milk Mixture

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ABSTRACT

The research aims to improve the therapeutic effect of white cheese by studying the effects of addition bee pollen on the bioactive behavior, sensory and physicochemical properties. Results indicated that bee pollen increased the antioxidant activities of the white cheese. The phenolic components in the bee pollen indicated good potential to inhibit the growth of *E. coli* (ACCT 8739), *St. aureus* (ATCC 6538) and *S. typhimurium* (ACCT 25566). Yield, total solid, fat and protein content of white cheese increased with the addition of bee pollen. No significant effects were observed on the sensory properties, compared with the control up to 1% (w/v). The addition of bee pollen during the production of white cheese is found to be necessary to increase the bioactive activities of the resultant cheese.

Keywords: bee pollen, polyphenols, physicochemical and sensory attributes, white cheese.

INTRODUCTION

The bee pollen has been collected by honey bees was used for human nutrition as a "perfect health food" after the Second World War (Bogdanov 2012). German Federal Board of Health, (Linskens and Jorde, 1997) recognized pollen as a medicine, because it contains bioactive components, which enhance health through the interactions of food matrix and nutrients in maintaining human biological processes (Saura-Calixto, F. 2011), such as proteins, carbohydrates, lipids, amino acids, minerals, vitamins (namely C, E, β carotene, B-complex), trace elements and polyphenols (Malerbo-Souza, 2011 and de Arruda *et al*., 2013). The main bioactive components of bee pollen are the polyphenols, mostly flavonoids. The main bioactive flavonoids are isorhamnetin3-*O*-rutinoside, quercetin3-*O*-rutinoside, naringenin, rhamnetin3-*O*-neohesperidoside, quercetin3-*O*-neohesperidoside, isorhamnetin, quercetin, kaempferol (Han, *et al*., 2012). Polyphenols possess diverse biological properties such as antiaging, anticarcinogen, antioxidant, antiinflammatory, cardioprotective and antiatherosclerosis, so the interest in the importance of polyphenols on human health has been growing (Han, *et al*., 2007).

Recently, cheese production containing nutritionally important amounts of polyphenols has increased because; cheese contains a small amount of polyphenols (O'Connell and Fox, 2001). Only a few researches have been proposed on the effects of phenolic compounds in rennet curds (Han, *et al*., 2011a and Han, *et al*., 2011b).Several researches aimed to improve the healthy effect of cheese, such as addition of the catechin to low-fat hard cheese (Rashidinejad *et al*., 2013). Sharma *et al*., (2011) produced a novel cheese with blending dried broccoli sprouts powder, and Giroux *et al*., (2013) in making Cheddar cheese enrichment with green tea polyphenols.

Camel milk is gaining increasing recognition due to it beneficial effects in the control and prevention of multiple health problems. Recent studies have shown that camel milk has anti-hypertensive, anti-carcinogenic, hepatoprotective, hypocholesterolemic effects as well as anti- diabetic and antioxidative factors (Majeed 2005; Elayan *et al*., 2008 and Khan and Alzohairy 2011 Abd Elhamid and Elbayoumi 2017), however, Mehaia and Qassim, (1993) indicated that the coagulum obtained from camel milk was very soft as compared to that of cow milk and took very long time, and he found that making the cheese from the mixture of camel milk and cow milk improved the coagulation process

The objective of this study was to examine the effect of the incorporation of bee pollen on the bioactive activities, sensory attributes and physicochemical properties of White cheese made from camel and cow milk mixture.

MATERIALS AND METHODS

Samples of bee pollen were collected from a commercial apiary located in Damanhour district, El-Behera governorate, Egypt. The collected pollen was dried at <40◦C for 20hrs. The dried pollen was grind and sieved through a 37 µm mesh, and stored in a freezer at −15◦C throughout the study.

(a) **(b)**

Figure 1. bee pollen (a): before grinding (b): after grinding

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programme was performed according to Oszmiansky, *et al*., (1988)

Cheese making was carried out by the method described by Abou-Donia (2008). Camel milk (0.25%) and fresh cow's milk (0.75%) were mixed. the mixture of milk was divided into five parts (5 kg), which were mixed with 0%, 0.5%, 1%, 1.5% and 2% (w/v) of bee pollen. Sodium chloride was added (3% w/w). Pasteurisation was carried out at 63°C for 30 min and then cooled to 42 °C, calcium chloride was added (0.02% W/V).Then inoculated with 2% of mixed (1:1) *Lb del* ssp. *bulgaricus* (Lb), and *S. thermophilus* (ST). Calf rennet was added to coagulate milk within 2–3 h. The curds were separately transferred into wooden frames and left 24 h for wheying off. The cheese

curd was cut into cubes (Figure 2), pickled in its whey (3% w/w salt), and stored for repining at refrigerated temperature (10°C) for 45 days. Three replicates of experimental cheeses were processed for bee pollen mixtures.

1 2 3 4 5

Figure 2. White cheese from camel and cow milk mixture with added bee pollen: 1, control without bee pollen; 2, white cheese with 0.5 g bee pollen / 100 ml milk; 3, white cheese with 1g bee pollen / 100 ml milk; 4, white cheese with 1.5 g bee pollen / 100 ml milk; 5, white cheese with 2 g bee pollen / 100 ml milk.

Bioactive Activities Analysis was examined by measuring the total phenolic content by the Folin-Ciocalteu method (Singleton, *et al*., 1999). For measuring the inhibition of ascorbate autoxidation, the method of Oyaizu (1986) was used, while reducing power assay was measured according to Zhu, *et al*., (2008).

Antibacterial activity test of the following strains: *Lb del* ssp. *bulgaricus* (EMCC 1102), and *S. thermophilus* (EMCC 1102), *St. aureus* (ATCC 6538), *B. cereus* (ATCC 9639), *S. typhimurium* (ACCT 25566) and *E. coli* (ACCT 8739), were used in the assay protocol (Collins, *et al*., 1995)

pH value of samples were determined using a digital pH meter as given by AOAC (2000), total solids contents were measured by the method of Marshall (1992), while the yield was calculated according to Sipahioglu *et al*. (1999).The total nitrogen- protein was measured using the Kjeldahl method (AOAC, 2000). Salt was determined using the method of Volhard (Jame 1995); and the fat according to AOAC (2000). All the above mentioned measurements were carried out in triplicate.The sensory evaluation of cheese treatments were evaluated according to Bodyfelt *et al*. (1988). Data were statistically analyzed using the statistical analysis system software package (SAS 2000). Analyses of variance were performed using ANOVA procedures. Standard error was obtained from the statistical model.

RESULTS AND DISCUSSION

Data of phenolic compounds by (HPLC) in bee pollen are shown in Table (1). Quercetin (251.21µg/g) was the major phenolic compounds detected in the samples with added of bee pollen, followed by the Ferulic (246.80µg/g). Similar result was obtained by Serra Bonvehí *et al*. (2001), who found that the predominant flavonoids was the quercetin (Bonvehí, *et al*.,2001). Bee pollen is also rich in *p*-coumaric (67.08µg/g), Kaempferol (49.22µg/g), Chrysin (25.14µg/g), Rutin (22.33µg/g) and Sinapic (20.67µg/g). Chlorogenic and Caffeic were present in very small amounts in bee pollen. These results were in agreement with Lundgren and Wiedenmann (2004) and Žilić *et al*., (2014).

For measuring the activity of antioxidant, results in Table (2) show that the total phenols increased from 11.53 to 46.12 mg/g cheese as the level of bee pollen increased from 0.5 to 2% at zero time. The inhibition of ascorbate content increased from 1303 to 5261 µg/g cheese. Similar observations were observed by Najgebauer-Lejko *et al.,* (2011) and Rashidinejad *et al*., (2013). Inhibition of ascorbate content increased significantly at the end of ripening period. This might be attributed to the presence of tyrosine residues, proteins or sugar components from lactose and oligosaccharide, being present in the cheeses (Singleton, *et al*., 1999). Reducing power assay (%inhibition) increased from 33.61 to 98.37 % cheese as the level of bee pollen increased from 0.5 to 2% at zero time. This result agreed with Rashidinejad *et al.,* (2013).

Table 1. Analysis of phenolic compounds by high-performance liquid chromatography (HPLC) in pollen bee.

Compound	Conc. (µg/g)	Compound	Conc. (µg/g)
Pyrogallol	ND	Rutin	22.33
Gallic	ND	*p*-coumaric	67.08
Protocatechuic	ND	Naringin	ND
p-hydroxybenzoic	14.85	Hesperidin	ND
Gentisic	ND	Apigenin-7-glucoside	ND
Cateachin	ND	Myricetin	ND
Chlorogenic	6.22	Rosmarinic	ND
Caffeic	11.86	Cinnamic	ND
Syringic	ND	Quercetin	251.21
Vanillic	ND	Apigenin	ND
Scopoletin	3.01	Kaempferol	49.22
Ferulic	246.80	Chrysin	25.14
Sinapic	20.67		

Table 2. Changes of total phenols content, inhibition of ascorbate and reducing power assay (%inhibition) contents in white cheeses from camel and cow milk mixture as affected by different bee pollen during repining storage.

Treatments	Storage period (days)	Total phenols (mg/g cheese)	Inhibition of ascorbate µg/g cheese)	reducing power assay (% inhibition)
Control cheese	0	1.44^{e}	139^{t}	3.61^{j}
	15	1.46^{e}	113^{s}	3.40^{j}
	30	1.49^{e}	100^{r}	3.22^{j}
	45	1.51^{e}	92^{q}	3.10^{j}
Cheese with 0.5% bee pollen	0	11.53^{d}	1303^{p}	33.61^{h}
	15	11.76^{d}	1297^{o}	32.72^{hi}
	30	12.00^{d}	1213^{n}	31.93^{hi}
	45	12.18^{d}	1157^{m}	30.83^{i}
Cheese with 1% bee pollen	0	23.05^{c}	2990^{l}	46.53^{f}
	15	23.45^{c}	2802^{k}	45.37^{f}
	30	23.74^{c}	2660^{j}	44.13^{f}
	45	23.94^{c}	2501^{i}	42.26^{g}
Cheese with 1.5% bee pollen	0	34.59^{b}	3172^{h}	76.75^{c}
	15	35.03^{b}	3131^{g}	75.36^{cd}
	30	35.36^{b}	3097^{f}	73.34^{de}
	45	35.68^{b}	3041^{e}	72.79^{e}
Cheese with 2% bee pollen	0	46.12^{a}	5261^{d}	98.37^{a}
	15	46.78^{a}	5117^{c}	97.21^{ab}
	30	46.33^{a}	5097^{b}	96.50^{ab}
	45	46.65^{a}	5071^{a}	95.43^{b}

***Means of triplicates. Means followed by the same superscript are not significantly different, P<0.05.**

Results in Table (3) indicate that there was no antibacterial effect on the *Lb. del.* ssp. *bulgaricus* (EMCC 1102) and *S. thermophilus* (EMCC 1102) up to 2% bee pollen, while in the case of *St. aureus* (ATCC 6538), the inhibition zones were 0.2,0.4,0.6 and 0.8 cm at 0.5,1,1.5 and 2% bee pollen ,respectively. Bee pollen showed no effect on *B. cereus* (ATCC 9639), which agreed with Erkmen and özcan (2008) and Yerlikaya (2014) at 2.5 % (v/v). The highest antibacterial activity was obtained with 2% bee pollen with a 1 and 1.2 cm zone of inhibition of *S. typhimurium* (ACCT 25566) and *E. coli* (ACCT 8739), respectively. Similar results were obtained by Yerlikaya (2014). This might be attributed to the presence of quercetin and other phenolic compounds in bee pollen, which are of antibacterial effect.

Table 3. Antibacterial activity (zone of inhibition: cm) of bee pollen.

strain	Bee pollen				
	control	0.5 %	1 %	1.5 %	2 %
Lb. del. ssp. *bulgaricus* (EMCC 1102)	_	_	_	_	_
S. thermophilus(EMCC 1102)	_	_	_	_	_
St. aureus (ATCC 6538)	_	0.2	0.4	0.6	0.8
B. cereus (ATCC 9639)	_	_	_	_	_
S. typhimurium(ACCT 25566)	_	0.3	0.5	0.9	1
E. coli (ACCT 8739)	_	0.3	0.8	1	1.2

***Means of triplicates. Means followed by the same superscript are not significantly different, *P*<0.05.**

pH value of White cheeses made with the addition of 0.5, 1, 1.5 and 2% pollen bee were significantly lower than the control treatment at zero time of repining period (*P*< 0.05(Figure 3). pH values increased after 45 days of repining period, which might be attributed to the fermentation of lactose by starter cultures (Faion *et al* 2015).Najgebauer-Lejko *et al*., (2011) who reported that the tea polyphenols were of a positive effect on yoghurt acidity. Similarly, the acidity produced from starter cultures with apple polyphenolic extracts in yoghurt was also observed by Sun-Waterhouse *et al*., (2012) and Rashidinejad *et al*., (2013).

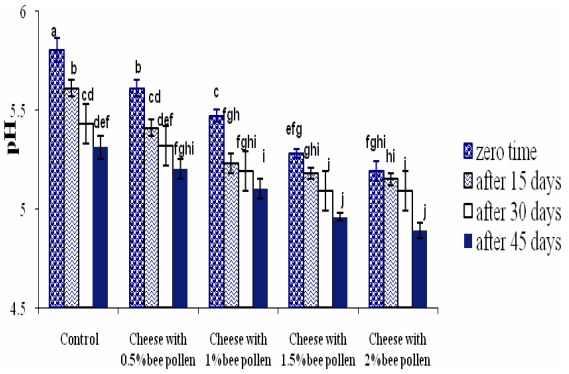

Figure 3. Changes in pH of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

Yield of cheese (Figure 4) increased from 22.1 to 24.6% as the level of bee pollen increased from 0.5 to 2% at zero time. This might be due to the addition of bee pollen, which is of high dry matter. Also, the addition of cow milk to camel milk improved the cheese yield as indicated with the control treatment, when compared with cheese yield camel milk found by Mehaia and Qassim, (1993).It was also observed that the yield of cheeses decreased during the repining period.

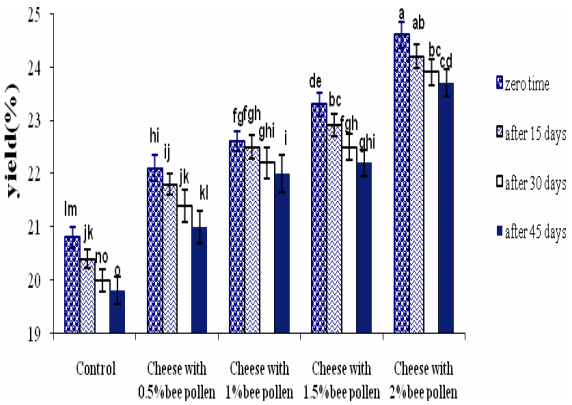

Figure 4. Changes in yield (%) of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

Statistical analysis (Figure 5) shows that the bee pollen is of a significantly positive effect (*P*<0.05) on the total solids (%). This might be due to the reduction of entrapped water in protein networks as a result of hydrophobic interaction between polyphenols and milk proteins, during the coagulation of cheese curd (Han *et al*., 2011). Also, the changes in the total solids of the samples were similar to those in the protein (Figure 6) and fat (Figure 7) and salt (%)(Figure 8), which did not change during the ripening period.

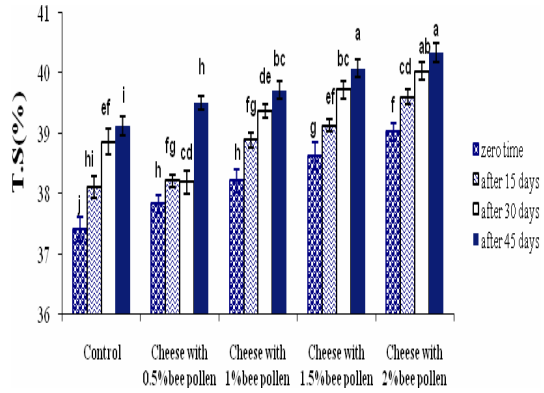

Figure 5. Changes in T.S. (%) of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

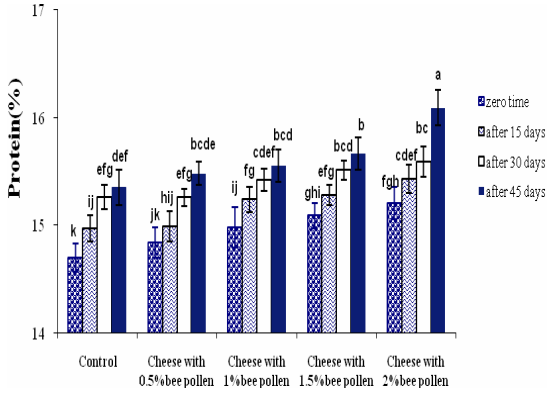

Figure 6. Changes in protein (%) of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

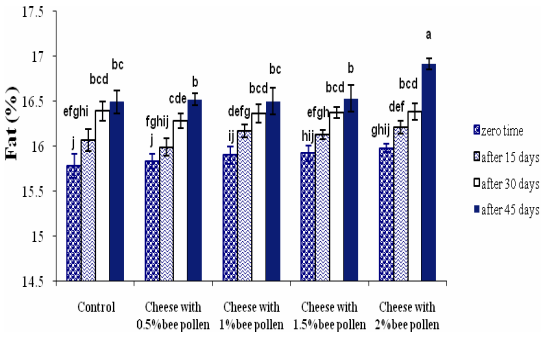

Figure 7. Changes in fat (%) of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

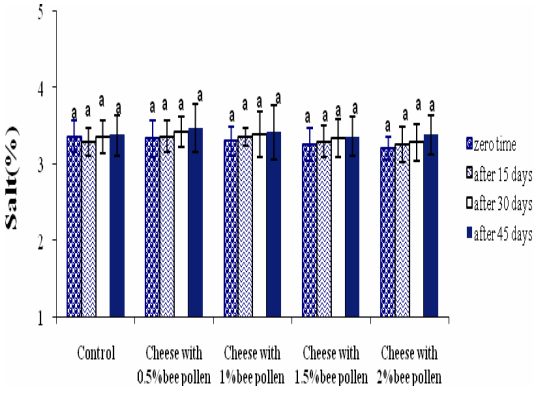

Figure 8. Changes in salt (%) of White cheese during ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

Regarding the sensory attributes, the addition of bee pollen effected significantly the body and texture, appearance, odour and flavour of the White cheeses due to the concentration of bee pollen(Figures 9 and 10). For example, the resultant cheeses with 1.5 and 2 % pollen bee characterized with the lowest scores for odour appearance, body and texture and flavour, while the addition of 0.5 and 1% bee pollen were of the highest scores for sensory properties of White cheeses. Faion *et al*., (2015) found that addition 0.2 wt. % of mate extract affected flavour, texture and acceptance of Prato cheese , which was of a bitter flavour after 45 days. Also, Sharma *et al*., (2011) obtained on acceptable cheese with 20% broccoli powder after freeze dried as a source of polyphenolics.

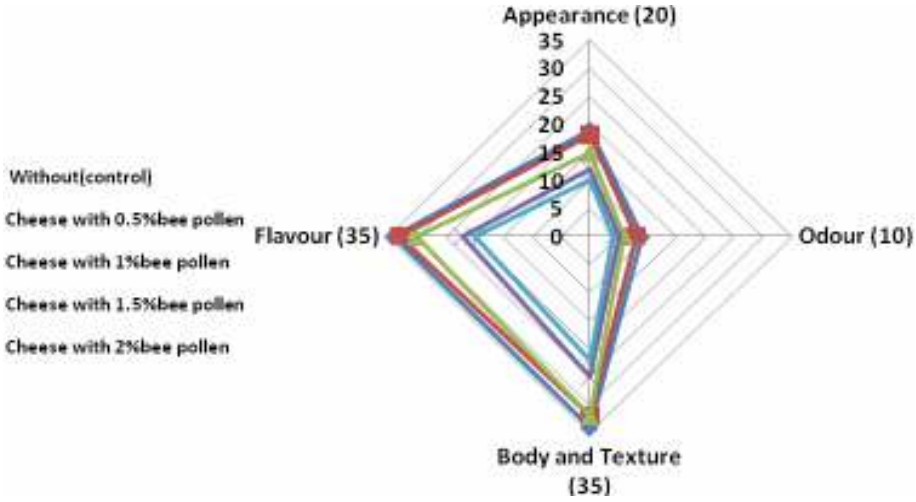

Figure 9. Sensory results for of White cheese at zero time. Error bars give the standard deviation, histograms labeled with different letters are significantly at *P*<0.05(Averages of three replicates).

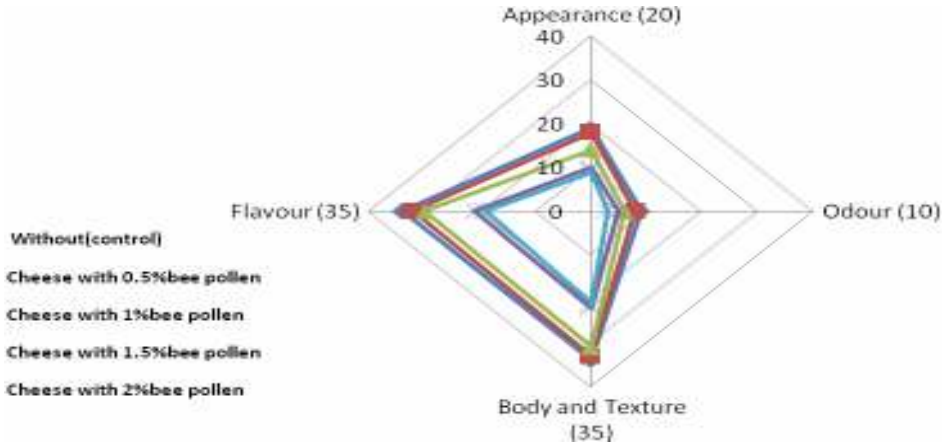

Figure 10. Sensory results for of White cheese after 45 days of ripening period. Error bars give the standard deviation, histograms labeled with different letters are significantly at P<0.05 (Averages of three replicates).

CONCLUSION

It could be concluded that by the addition of bee pollen to white cheese made from camel milk and cow milk mixture up to1% was positive effect on the level of bioactive behavior as indicated by the total phenolic compounds, antibacterial and antioxidant activities, and did not have effect on the sensory attributes.

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تأثير حبوب اللقاح على سلوك النشاط الحيوى و الخصائص الحسية و الفيزيوكيميائية للجبن الأبيض المصنع من خليط من لبن الابل ولبن الابقار

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يهدف البحث الى تحسين التأثير العلاجى للجبن الابيض، حيث تم دراسة تأثير إضافة حبوب اللقاح على سلوك النشاط الحيوى و الخصائص الحسية و الفيزيوكيميائية للجبن الأبيض، و أظهرت النتائج أن إضافة حبوب اللقاح أدى الى زيادة النشاط المضاد للاكسدة للجبن، كما أسفرت ان المركبات الفينولية فى حبوب كان لها تأثير قوى على إيقاف نمو هذه السلالات على الاطباق (ACCT 8739), *St. aureus* (ATCC 6538) *S. typhimurium* (ACCT 25566). *E. coli* ، و أيضا أظهرت النتائج ان نسبة التصافى و الجوامد الصلبة و الدهن للجبن زادت بزيادة نسبة حبوب اللقاح مقارنة بالكنترول ، و قد إتضح من التقييم الحسى إنه يمكن إنتاج جبن أبيض بإضافة حبوب لقاح حتى نسبة ١%(وزن / حجم) دون تأثير على الخصائص الحسية. لذلك إضافة حبوب اللقاح أثناء إنتاج الجبن الابيض يكون ضرورى لزيادة النشاط الحيوى للجبن.