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Processed Cheese Sauce Functionalized with Microencapsulated Fig Leaves Extract

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Abstract

In the current study, microcapsules with *Lactobacillus helveticus* with or without fig leaves extract were performed. The effects of fig leaves extract on probiotic strains; antioxidant/antimicrobial activities were studied. The morphological characteristics and efficiency of microcapsules were determined. The fig leaves extract possess total phenol content, antioxidant activity, and excellent antimicrobial properties against pathogens. All tested probiotics propagated well when using fig leaves extract especially *with Lb. helveticus.* The encapsulation efficiency was recorded 92.00 and 92.12 % for capsules form with alginate and skim milk, respectively. The impact of supplemented different microcapsules with cheese sauce on microbiological, physicochemical, melting, oil separation, coloring, and sensory properties were assessed during 50 days of cold storage. Supplemented cheese sauce with microcapsules loaded by *Lb. helveticus* with fig leaves extract eliminated molds and yeast growth and prolonged the shelf life. This supplemented improved physiochemical, melting, and sensory properties during the storage period.

Keywords: Fig leaves extract; antimicrobial activity; antioxidant properties; microencapsulation; cheese sauce.

Introduction

Fig (*Ficus carica* Linn.) is a tree of small lengths that belongs to the family Moraceae, which is known as one of the oldest plants in the world. Ficus species are used as food or medicinal agents to enhance human health. Different parts of the plant such as bark, leaves, seeds fruits, and latex are medicinally vital [1]. Obtained fig leaves extract were reported in diabetic rats as antioxidants [2], in humans as inhibitors of LDL oxidation [3], and as inhibitors of several cancer cell lines [4]. Furthermore, it lowers the levels of triglycerides, total cholesterol and has been used as a medicine for the liver and kidneys [5-7].

Besides, the ethanol extract of fig leaves displayed higher antimicrobial activity against both Gram-negative and Gram-positive bacteria such as *Escherichia coli, Salmonella typhimureum, Bacillus cereus,* and *Staphylococcus aureus.* Moreover, the ethanol leaves extract provided good antifungal activity against *Candida albicans* and *Aspergillus niger* [8]. Additionally, the strong antimicrobial activity of fig leaves extract could be owed to the presence of phytochemicals and phenolic compounds in the leaves extract such as flavonoids, terpenoids, saponins, tannins and phenol [9, 10]. So, the addition of fig leave extract to food products gave healthy effects and played as preservative agents especially in the rapid spoilage dairy products.

Consumers in last year have become attractive to consume healthy foods to protect themself from illness. Probiotic bacteria are becoming used as food cultures, equivalent to amplified awareness of their impact on good health. It has been mentioned that foods fortified with probiotic bacterial counts not at least 10^7 CFU / gram at the period of consumption, to benefit the consumer [11, 12]. Dried preparations of probiotic cultures are a valuable method for preservation to a long period and are facilitated to add to functional products. The maintenance of viability during the drying process is the challenge to applied probiotic cultures in functional products. The major factor that influences on the survivability and stability of probiotic cultures during storage is the processing problems during drying of bacterial cells.

Microencapsulation can keep these sensitive cells against adverse environments like oxygen,

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acidic and temperature levels, freezing and during passage through the gastrointestinal tract of consumers [13-15]. There are diverse methods of microencapsulation as an emulsion, extrusion, spray-drying, and freeze-drying methods. During the freeze-drying method, porous are creating from the ice crystals through the freezing stage, which frequently leads to good rehydration performance of the powdered product [16]. Johnson and Etzel [17] studied the viability of *Lactobacillus helveticus* during spray drying and freeze-drying methods and they found the greater number of viable bacterial counts were present in the freeze-dried product.

To the best of our knowledge, there are no previous studies used the microencapsulation formula containing probiotic bacteria and fig leaves extract by freeze drier technique. Additionally, processed cheese sauces are consider a novel product in the Egyptian market although being widely used in the food sectors in pre-prepared and equipped fast meals as a desirable appetizer ingredient. Therefore, the present study was designed to confirm the efficacy of fig leaves extract as an antimicrobial agent and to enhancement the viability of the probiotics. Additionally, the production of functional cheese sauce as the dairy product model to apply the different formula of encapsulated fig leaves extract with *Lactobacillus helveticus*. The changes in chemical, physical, microbiological, and sensory characteristics of cheese sauce treatments were detected during the storage period.

2. Materials and Methods

2.1. Materials

Cheddar cheese was obtained from the local market, Cairo, Egypt. Corn starch was gotten from the starch and glucose company, Cairo, Egypt. Commercial fine grade salt was obtained from El-Nasr saltines Co. Alexandria, Egypt. Emulsifying salts were obtained from JOHA BK Ladenburg Corp, GmbH, Germany. Skimmed milk powder was obtained from the local market and sodium alginate was obtained from Sigma Chemical Company. Fresh fig leaves were collected from farms in the Menofya government in May 2019, Cairo.

2.2.1. Preparation of fig leaves extract

Fig leaves were washed and shade dried for 7 days at room temperature and then cut and milled to the powder with a special electric grinder (Moulinex). 100g of dried leaves were extracted with 500 ml of 90% ethanol (v/v) by cold extraction, left for 3 days at room temperature, and then filtered through No.1 filter paper [18]. The extracted mixture was evaporated under reduced pressure to dryness using a rotary evaporator at 40°C. The dried extract was then stored in dark sterile screw-capped bottles at 4°C until required for the use.

2.2.2. Antimicrobial assay by disc diffusion methods

One ml of leaves extract diluted in DMSO (dimethyl sulfoxide) to obtain 250, 200, 150, 100, 50 mg /ml. The different sterile paper discs were saturated with 20µl of each leaves extract concentration and sited on the surface of nutrient agar inoculated with the test microbe (10^5 CFU/mL) using swab. According to this method, each disc loaded with leaves extract equals to 1.00, 2.00, 3.00, 4.00, and 5.00 mg extract. The inoculated plates were incubated at the suitable temperature for each test microbe (37ºC for the bacteria and 30ºC for the fungi) for 24h. Leaves extract concentration was tested against different microbial strains: i.e. *Bacillus cereus* B-3711, *Bacillus subtilus* B-14472, *Aspergillus flavus* B-3357 (Northern Regional Research Laboratory Illinois, USA NRRL). *Listeria monocytogenes* 598 (University of Massashusetts, Ambert MA, USA). *Escherichia coli* 9637, *Salmonella typhimurium* 14028s, and *Staphylococcus aureus* 8325 (collected from National Research Center). *Yersinia enterocolitica* 10938, *Pseudomonas aeruginosa* 9027, and *Aspergillus niger* 3858a (Hungarian National Collection of Medical Bacteria, Hungary). The inhibition zones were evaluated as a diameter in mm [19].

2.2.3. Effect of leaves extracts on probiotic cultures

Different lactobacilli strains were individually added and grown in MRS broth medium fortified with 1.00, 1.50, 2.00, 3.00, and 4.00 mg/ml medium of leaves extract at 37ºC using 2% inoculums and incubated for 24h. The count of different lactobacilli strains was recognized by the MRS agar medium and incubated for 48 h at 37ºC [19]. The tested lactobacilli strains *Lb. planatrum* 4496, *Lb. casei* FEGY 9973, *Lb rhamnosus* 306 and *Lb. helveticus* CNRZ 32 were collected from microbiological Lb. Dairy Department, National Research Centre, Egypt.

2.2.4. Total phenol content (TPC) for the fig leaves powder

Total phenol content of fig leaves powder was determined calorimetrically at 625 nm by the Folin–Ciocalteau reagent according to the method described by Rashidinejad et al. [20].

2.2.5. Antioxidant assay for fig leaves powder

The antioxidant activity of the phenol extracts was estimated by using the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to the modification method by Bandoniene et al. [21].

2.2.6. Microencapsulation of *Lb. helveticus* with leaves extract using freeze-drying method

2.2.6.1. Growth condition of microbial culture

The *Lb. helveticus* strain was activated to obtain high biomasses using MRS broth and incubated for 24 h at 37 ºC., the cell pellets were harvested by centrifugation at 5000 rpm, for 15 min at 4ºC. The pellets were washed by a sterile saline solution (0.9% (w/v) NaCl) and stored at 8ºC to be encapsulated.

2.2.6.2. Microencapsulation procedure

The cells of *Lb. helveticus* was mixed with Skimmed milk (15% W/W) or sodium alginate (3%W/W). The other part of cells encapsulated using skimmed milk or sodium alginate with added fig leaves extract at the ratio of 10 mg/g cells in order to obtain desired core-to-wall ratios of 1:3. The four mixtures were stirred well by magnetic starrier and subjected to Freezer drier for 8 h and the obtained microcapsules freeze-dried powder was stored at 4ºC [22].

2.2.6.3. Encapsulation efficiency

Encapsulation efficiency (EE) was determined by using the following equation as described by Fareez et al. [23]:

$$EE = \frac{\text{Log}_{10} N}{\text{Log}_{10} N0} \times 100$$

Where N is the number of the bacterial cells loaded inside the microcapsules and No is the number of the free bacterial cells before microencapsulation.

2.2.6.4. Morphological characterization of microcapsules forms

The morphology of the microcapsules forms was examined using a scanning electron microscope (SEM) (JSM 6360LV, JEOL/Japan). Previously, the different microcapsules were added in buffer glutaraldehyde (0.1 M) at 4 ºC for 2 h and then were post-fixed with osmium tetraoxide (0.1 M) at 4 ºC for 1 h. Microcapsules were then continuously dried using 30, 50, and 70% ethyl alcohol for 2 min each and stayed in 100% ethyl alcohol for 30 min at 4ºC. After that, the microcapsules were sited on a piece of adhesive paper and covered with gold using a vacuum sputtering coater (Edwards S15) [13].

2.2.7. Preparation of functional cheese sauce

In this study, the formulation of cheese sauce was described by Li et al. [24]. Water was heated to 82–93ºC in a kettle, disodium phosphate, and sodium citrate were added with agitation, followed by the sliced cheese and the emulsifier. High shear mixing was sustained until the cheese was completely melted. Starch and salt were then added to the blend with high agitation. The sauce was then placed into stainless steel containers and stored overnight at 7ºC for processing the next day. The sauce was heated in a kettle to about 100ºC with agitation until it was uniformly melted without chunky particles. The cheese sauce was divided into six treatments and added free cells or encapsulated formula at a concentration of 1%.

Treatment 1, was served as a control contented free cells of *Lb. helveticus*

Treatment 2, was served as a control contented free cells of *Lb. helveticus* and fig leaves extract

Treatment 3, was contented microcapsules formula with *Lb. helveticus* by sodium alginate

Treatment 4, was contented microcapsules formula with *Lb. helveticus* and fig leaves extract by sodium alginate

Treatment 5, was contented microcapsules formula with *Lb. helveticus* by skim milk

Treatment 6, was contented microcapsules formula with *Lb. helveticus* and fig leaves extract by using skim milk

All treatments were purred into polyethylene cups (50 g) and capped directly after filling. The resultant cheese sauces were cooled at room temperature then stored at the refrigerator at 6± 1°C. The experiment was carried out in triplicate. Data were reported as the average of three independent

2.2.7.1. Chemical analysis

Samples were analyzed for total solids (T.S), fat, titratable acidity (T.A), and ash contents as mentioned by AOAC [25]. Total Volatile Fatty Acids (TVFA) value was determined according to Koiskowski [26] and values were stated as ml of 0.1 N NaOH/100 g cheese.

2.2.7.2. Physicochemical properties

pH Values were measured by the electric HANNA instrument pH 213 microprocessor pH meters by inserting the pH combined glass electrode directly in the sample.

2.2.7.3. Physical properties

Meltability of cheese sauces samples was determined according to the method designed by Olson and Price [27] as modified by Savello [28]. Oil Separation Index (OSI) of cheese sauces was determined as described by Thomas [29].

2.2.7.4. Microbiological analysis for functional cheese sauce during storage

Cheese sauce treatments (25 g) were homogenized for 1 min with 225 ml of tri-sodium citrate (2% w/v) as a sterile solution. Decimal dilutions were prepared in saline and the viable microbial counts checked at an interval of 10 days over a period of 50 days of storage. Viable microbial counts as log Colony Forming Units/g (log CFU/g) were enumerated by plate count method as follows: *Lb. heveticus* either free or encapsulated form in the final product was checked using the MRS agar medium for pouring the plates and incubated anaerobically at 37°C for 48 h [30]. Yeast and mold counts were detected using potato

dextrose agar (pH 3.5) according to the method of APHA [31]. The plates were incubated aerobically at 25°C for 3 - 5 days. Coliform groups were detected according to FDA [32] using violet red bile Agar (Difco) and the plates were incubated at 35 ºC for 24 hrs.

2.2.7.5. Color measurements

Color values of cheese sauce samples were measured using a Hunter colorimeter model D2s A-2 (Hunter Assoc. Lab Inc., VA, USA) according to El-Sayed [33].

2.2.7.6. Sensory evaluation

The evaluation of sensory properties was done when fresh and during 50 days of the cold storage period, according to the scheme of Meyer [34] by regular scoring panel members of the Dairy Department, National Research Center (NRC).

2.2.7.8. Statistical analysis

The data were analyzed according to Statistical Analysis System Users Guide SAS [35], (SAS Institute, Inc., USA). Separation among means in triplicate was carried out using Duncan multiple tests.

3. Results and Discussions

3.1. Total phenolic content and antioxidant activity of fig leaves powder

Before the preparation of fig leaves extract, Total phenol content and antioxidant activity are evaluated and shown in **Table 1**. It is seen that the total phenol compound of fig leaves powder was 8.54 ± 4.53 mg/g dry matter, which can be revealed mainly to the presence of phytochemical compounds in this leaves [36]. Also, the antioxidant activity of fig leaves powder was 48.17% ±1.93, which attributed principally to the phenolic content of them [6-37]. Therefore, fig leaves can be considered a good source of polyphenols and had beneficial action as an antioxidant that can be deactivated by free radicals to prevent therapy diseases resultant from oxidative damage of human cells [38].

Table1. Total phenol content and antioxidant activity by DPPH for fig leaves powder

Plant	Total phenol content (mg/g dry leaves)	Antioxidant activity, %
Fig leaves powder	8.54 ± 4.53	48.17% ±1.93

Mean± standard deviation

3.2. Antimicrobial assay by disc diffusion method

Figure 1 shows the susceptibility of pathogenic strains to different concentrations of fig leaves extract as determined by the disc diffusion method. Our results indicated that the fig leaves extract have strong activity on tested strains by detected inhibition zone and able to control microbial contamination. By way, the concentration 5.00 mg/ml was found significantly more effect on all tested strains, which the high inhibition zone recorded for *B. subtilus* with 29.66 mm, followed by *Y. enterocolitica* with inhibition zone 20 mm. Furthermore, the lowest influence was detected on *L. monocytogenes* and *A. niger* (11 mm). Moreover, the low concentration (1.00 mg /ml) has an antimicrobial effect against all tested pathogens, in which the value of the inhibition zone ranged between 4 to 11 mm. From earlier studies, it was established that the antimicrobial influence is due to the presence of alkaloids, flavonoids, coumarins, saponins, and terpenes [39,40] and various phenolic compounds by pharmacological activity have previously been separated from fig leaves [41,-42].

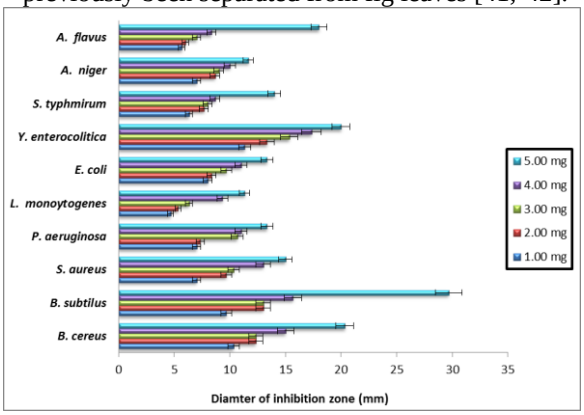

Fig.1. Antimicrobial assay of fig leaves extract by disc diffusion method

3. 3. Effect of fig leaves extract on probiotic strains

The effect of fig leaves extract at varied concentrations on the growth of probiotics bacteria is presented in **Fig. 2.** The growth of all studied probiotic strains was affected by different concentrations. The obtained data showed that increasing the concentration of different extracts from control to 5.00 mg/ml medium significantly led to raising the probiotic bacterial growth. Also, it could be recognized that all studied probiotic bacteria significantly propagated well when using fig leave extract. The significantly viable counts observed for *Lb. helveticus* and *Lb. rhamnosus*, which the viable counts increased about 0.57 and 0.50 log cycles for the same strains respectively at the concentration 5.00 mg/ml. But the viable count of *Lb. casei* and *Lb. planatrum* increased only 0.4 and 0.23 log cycles, respectively at the concentration of 5.00 mg/ml. Our data indicated that the fig leaves extract not negatively affected the probiotic strains and able the kept bacterial growth. This optimistic influence can be attributed to the antioxidant action of the fig leaves extract

[44, 45]. From these results, we used *Lb. helevticus* either capsules or free cells from in manufacturing of Functional sauce cheese.

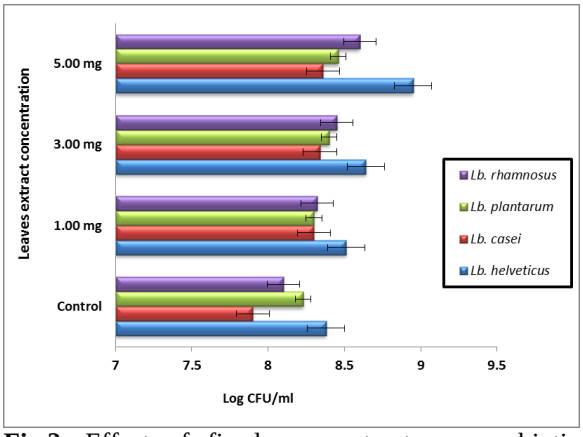

Fig.2. Effect of fig leaves extracts on probiotic bacteria Log CFU/ml

3.4. Encapsulation efficiency (EE)

The microcapsules efficiency of the capsules loaded with *Lb. helveticus* was recorded 94.21 % and 94.60 % for capsules form with alginate and skim milk, respectively and fig leaves extract (10 mg/g cells). Moreover, the microcapsules efficiency of capsules loaded with *Lb. heveticus* was recorded 92.00 and 92.12 % for capsules from with alginate and skim milk, respectively. According to our results, there was a slightly significant difference in the microcapsules efficiency between the microcapsules materials loaded with *Lb. helveticus.* This result could be considered to the mild encapsulation method used for producing all capsules regardless of the encapsulating material [13, 46]. Also, found fig leaves extract able to preserve the viability of *Lb. helveticus* during the freeze drier process. Many authors informed that polymer concentration could affect the encapsulation yield. Sultana et al. [47] indicated the addition of Hi-maize resistant starch in alginate beads could enhance the encapsulation efficiency of *Lb. casei*. Sandoval-Castilla et al. [48] reported that the microencapsulation yield for *Lb. casei* varied from 50.9% to 80% depending on the concentration of polymer using in the microencapsulation technique.

3.5. Morphological characterization of microcapsules forms

The morphology of the freeze-dried microcapsules loaded with *Lb. helveticus* with or without fig leaves extract by scanning electron microscopy (**Fig. 3**) indicated high accumulation, irregular in a spherical shape, surface, and creating a diversity of sizes regardless or elongated of the treatments. However, at higher magnification (20000 & 8000) for microcapsules form with skim milk (**Fig. 3 C & D**), some fragments were noticed, with not detected spherical shape and the *Lb. helveticus* cells are occurrence. Moreover, it indicated some porous matrix in the morphological shape of microcapsules forms, resulting from dehydration of freeze-dried of polysaccharide. Additionally, microencapsulation using freeze drier technique is progressing at low temperature lead to the formation of ice crystals and under high pressure, resulting in the formation of porous dry products [13, 22, 49].

3.6. Chemical composition of functional cheese sauce

Table 2 shows the chemical composition of cheese sauce; total solids ranged from 32.39 to 34.86 %. Total solids significantly increase and moisture decreased as a function of added materials of microcapsules probiotic bacteria or/and fig extract. This result agrees with **Desouky** *et al.* [50]. The fat content of processed cheese sauce significantly increased (0.50%) in probiotic or/and fig extract microcapsules treatments. The increase of fat content with the addition of microencapsulation materials is related to an increase in total solids. The fat/DM was significantly highest with control (T1) is also related to its fat and total solids content. Protein content was ranged from 8.70 to 9.95%, the highest protein content observed with T2 (9.95%) that made by the addition of fig extract and free cell. This may be due to the chemical composition of fig leaves extract [19].

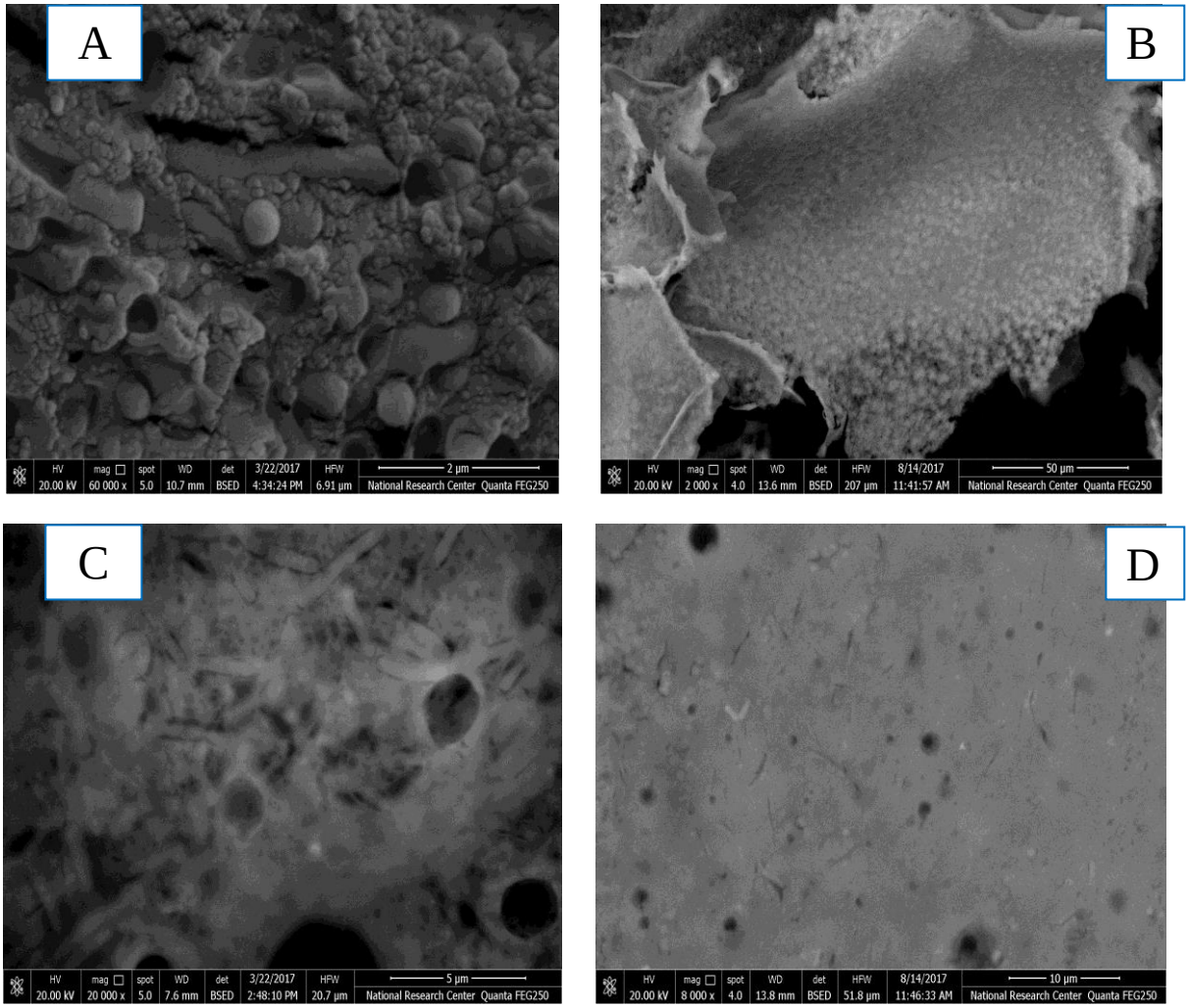

Fig.3. Morphological characterization of capsules forms, (A) Microcapsules by sodium alginate loaded with *Lb. heveticus*, (B) Microcapsules by sodium alginate loaded with *Lb. heveticus* + fig leaves extract, (C) Microcapsules by skim milk loaded with *Lb. heveticus*, (D) Microcapsules by Skim milk loaded with *Lb. heveticus* + fig leaves extract.

Table 2. Chemical composition of functional cheese sauce

Treatment	T.S	Moisture	Fat	Fat/DM	Protein	Ash
T1	32.39^{F}	67.61^{A}	15.00^{B}	46.31^{A}	9.10^{C}	3.30^{D}
T2	32.98^{E}	67.02^{B}	15.00^{B}	45.48^{D}	9.95^{A}	3.44^{B}
T3	34.35^{B}	65.65^{D}	15.50^{A}	45.12^{E}	9.66^{B}	3.19^{E}
T4	33.70^{D}	66.30^{C}	15.50^{A}	45.99^{C}	8.72^{D}	3.46^{A}
T5	34.86^{A}	65.14^{E}	15.50^{A}	44.31^{F}	9.71^{B}	3.39^{C}
T6	33.68^{C}	66.32^{C}	15.50^{A}	46.02^{B}	8.70^{D}	3.29^{D}

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$. T1: Control (cheese sauce), T2: Cheese sauce with free cells + fig leaves extract, T3: Cheese sauce with microcapsules with alginate, T4: Cheese sauce with microcapsules by alginate + fig leaves extract, T5: Cheese sauce with microcapsules by skim milk, T6: Cheese sauce with Microcapsules by skim milk + fig leaves extract.

Table 3. Chemical compositions of functional cheese sauce fresh and during cold storage for 50 days

Components	Storage (days)	T1	T2	T3	T4	T5	T6
T.S	Fresh	32.39^{Fd}	32.98^{Ed}	34.35^{Bd}	33.70^{Cf}	34.86^{Ad}	33.68^{Df}
	10	33.04^{Fc}	33.57^{Ec}	34.82^{Bc}	34.45^{De}	35.77^{Ac}	34.61^{Cd}
	20	34.97^{Cb}	33.87^{Fb}	36.37^{Ab}	34.59^{Dd}	35.91^{Bb}	34.45^{Ee}
	30	36.55^{Ca}	34.47^{Fa}	39.39^{Aa}	35.16^{Dc}	38.05^{Ba}	34.69^{Ec}
	40	-	-	-	35.21^{Ab}	-	34.88^{Bb}
	50	-	-	-	36.05^{Aa}	-	34.96^{Ba}
Fat	Fresh	15.00^{Bc}	15.00^{Bc}	15.50^{Ab}	15.50^{Ac}	15.50^{Ac}	15.50^{Ae}
	10	15.50^{Bb}	15.50^{Bb}	15.50^{Bb}	15.50^{Bc}	16.00^{Ac}	16.00^{Ad}
	20	16.00^{Ba}	16.00^{Ba}	16.00^{Ba}	16.00^{Bb}	16.50^{Ab}	16.50^{Ac}
	30	16.00^{Ba}	16.00^{Ba}	16.00^{Ba}	16.00^{Bb}	17.00^{Aa}	17.00^{Ab}
	40	-	-	-	16.00^{Bb}	-	17.00^{Ab}
	50	-	-	-	16.50^{Ba}	-	17.50^{Aa}
pH	Fresh	6.67^{Aa}	6.47^{Da}	6.50^{Cb}	6.02^{Fa}	6.57^{Ba}	6.14^{Ea}
	10	6.42^{Cb}	6.30^{Db}	6.54^{Aa}	5.99^{Fb}	6.49^{Bb}	6.13^{Ea}
	20	6.32^{Bc}	6.09^{Ec}	6.40^{Ac}	5.97^{Fc}	6.20^{Cc}	6.12^{Da}
	30	6.21^{Bd}	5.99^{Dd}	6.33^{Ad}	5.94^{Ed}	6.07^{Cd}	6.06^{Cb}
	40	-	-	-	5.90^{Be}	-	6.00^{Ab}
	50	-	-	-	5.87^{Bf}	-	5.98^{Ac}
T.A	Fresh	1.20^{ABb}	1.23^{ABd}	1.16^{BCd}	0.92^{Df}	1.12^{Cd}	1.25^{Af}
	10	1.22^{Cab}	1.25^{Bc}	1.20^{Dc}	1.12^{Fe}	1.16^{Ec}	1.28^{Ae}
	20	1.25^{Cab}	1.28^{Bb}	1.23^{Db}	1.16^{Fd}	1.20^{Eb}	1.30^{Ad}
	30	1.28^{Ba}	1.31^{Aa}	1.25^{Ca}	1.20^{Ec}	1.23^{Da}	1.32^{Ac}
	40	-	-	-	1.24^{Bb}	-	1.35^{Ab}
	50	-	-	-	1.27^{Ba}	-	1.38^{Aa}
TVFA (ml) 0.1 N NaOH /100g	Fresh	9.20^{Cd}	10.00^{BCd}	11.00^{ABc}	12.00^{Ae}	10.00^{BCd}	12.00^{Af}
	10	11.00^{Cc}	13.00^{ABc}	13.00^{ABb}	13.00^{ABd}	12.00^{BCc}	14.00^{Ae}
	20	12.50^{Cb}	14.00^{Cb}	14.00^{Cb}	14.00^{Cd}	16.00^{Bb}	18.00^{Ad}
	30	15.00^{Ca}	15.70^{Ca}	16.00^{Ca}	18.00^{Bc}	18.00^{Ba}	21.00^{Ac}
	40	-	-	-	21.00^{Bb}	-	25.00^{Ab}
	50	-	-	-	24.00^{Ba}	-	28.00^{Aa}

Data expressed as mean of three replicates. Means between rows showing the same capital letters are not significantly different ($p \leq 0.05$). Means between columns showing the same small letters are not significantly different ($p \leq 0.05$). T1: Control (cheese sauce), T2: Cheese sauce with free cells + fig leaves extract, T3: Cheese sauce with microcapsules with alginate, T4: Cheese sauce with microcapsules by alginate + fig leaves extract, T5: Cheese sauce with microcapsules by skim milk, T6: Cheese sauce with Microcapsules by skim milk + fig leaves extract.

3.7. Chemical compositions of functional cheese sauce fresh and during cold storage

Data presented in **Table 3** refers to the chemical composition of functional cheese sauce fresh and during cold storage up to 50 days. Total solids significantly increase in all treatments and during cold storage. The total solids were higher in the treatments that made by microencapsulation material or/and fig extract compared with the control, this may be due to binding properties of encapsulation materials (alginate and skim milk powder) used in this study. These results confirmed by **Desouky et al.** [50] who found that the substitution of cheddar cheese with camel milk powder at different ratios increased the total solids content. Fat content significantly increased in treatments and after storage for 20 days. The highest fat content recorded with T5 (17.00%) and T6 (17.50%) at the end of storage (50 days). The incensement in fat content is also due to an increase in total solids content [51, 52].

The pH values significantly decrease within treatments as well as during storage, in contrast to the acidity values that are increasing in treatments

as well as storage. The changes in acidity within treatments and during storage could be due to the changes occurring in total solids, protein content, and emulsifying salt form as reported by **Youssef et al.** [53]**.** It can also be caused by the activity of free and microcapsules probiotic bacteria [19].

Total volatile fatty acids (TVFAs) are also presented in **Table (3)**. As noted, the values of TVFAs significantly increased in treatments by adding free and/or microcapsules cells compared to the control in the presence or absence of fig leaves extract. The highest TVFAs content recorded with T6 (12.0) and the lowest value was in T1 (9.2) in fresh time. After storage for 50 days, TVFAs significantly increased in all tested cheese, T6 gained the highest value (28) followed by T4 (24). The changes in TVFAs during storage could be due to the activity of free or/and *Lb. helveticus* added to treatments [19, 54]. Also, the enhancement of TVFAs content in treatments contains fig leaves extract due to high phenolic compounds, organic acids, and volatiles in fig leaves as reported by Gani et al. [55].

The total solids, fat, pH, TA, and TVFAs have not detected more than 30 days into other treatments (T1, T2, T3, and T5) that become spoilage (yeast & molds) after 30 days of storage as mentioned in paragraph (3.10). The addition of fig leaves extracts to processed cheese sauce manufactured extended the shelf life of cheese to 50 days as confirmed by microbiological evaluation as shown in (Table 6).

3.8. Melting changes of functional cheese sauce

Table 4 showed the values for the melting of different treatments of functional sauce cheese fresh and after 50 days of cold storage. Data were recorded as functional sauce cheese flow [mm]. Data pointed to that the melting values of all functional sauce cheese treatments increased as the storage period progressed. Furthermore, the melting index decreased in functional sauce cheese treatments which content microcapsules extract and *Lb*. *helveticus* using alginate and skim milk after 50 days of cold storage (T4 & T6), compared with treatments without addition extract to its cheese base formula after 30 days of cold storage (T3 & T5), which may be owed to the high carbohydrate content in freeze-dried fig extract and can be active as a stabilizer and bind water which increase the viscosity. The data are in the same line as that of El-Dardiry et al. [56]. Contrary to that, the melting index increased in the control treatment (T2) which content extract without microcapsules when compared with control free (T1), which may be due to the moisture or total solids content in these treatments as confirmed by Desouky et al. [50]. In general, the highest melting values observed in control treatments T1 and T2 after 30 days of cold storage (32 and 39 mm) respectively. The lowest melting values noted in T4 and T6 after 50 days of cold storage (20.5 and 21.5mm) respectively.

3.9. Changes of oil separation index (OSI) in functional cheese sauce

Table 5 explained the changes for the OSI values of fresh functional cheese sauce and after 50 days of cold storage. The OSI of functional cheese sauce was affected by microcapsules loaded with extract plus *Lb*. *helveticus* strain using alginate or skim milk and storage period, the OSI was increased significantly in (T4 & T6) which content capsules fig leaves extract after 50 days of cold storage compared with T3 and T5 without adding capsules extract Also, control treatments took the same way that the OSI increased significantly in control which content fig leave extract in free form (T2) than another control treatment not including extract (T1). Moreover, the OSI values of all cheese treatments increased as the storage period progressed. The highest OSI values were observed in T6 which content microcapsules extract and *Lb*. *helveticus* using skim milk after 30 and 50 days of cold storage (0.86 and 1.49) respectively. The increase in OSI in the stored Cheese sauce and in the samples content extracts could be related to the increase of the acidity and more degradation of protein contents in these samples which affects a higher fat leakage. These data are in agreement with the findings of Awad et al. [57] and Dardiry et al. [56].

3.10. Microbiological analysis for functional cheese sauce during storage

In this study, the effect of fig leaf extract was evaluated on the enumeration of the encapsulated and free cells of *Lb. helveticus* after added to functional cheese sauce as Table 6. Generally, the *Lb. helveticus* count in all treatments decreases during the storage period for 50 days. In addition, it was found that adding fig leaves extract enhanced the viability of bacterial strain, especially in the microcapsules form (T4 & T6). Our results indicated the microcapsules form able to protect the bacterial counts during long storage as mentioned before [11, 13, 14, 58]. Also, the presence of fig leaves extract inside capsules significantly improved the bacterial count more than control (T1) during the storage period. The number of bacterial cells reached 6.55 log CFU/g for free cells form (T1) but the overall count improved to 9.20 and 8.99 log CFU/g for encapsulated form with alginate and skim milk (T4 & T6, respectively) at 30 days of storage. Moreover, the counts inside microcapsules form without leaves extract reached to 8.56 & 8.77 log CFU/g (T3 & T5, respectively) compared with

free cell form (T1) at 30 days of storage. The presence of phenolic compounds and antioxidant activity were encouraged the viability of *Lb. helveticus* during storage periods for cheese sauce [19, 59, 60].

Our results also, observed that the treatments free from extract became moldy and not acceptable, in which the count of molds and yeasts reached 3.00, 2.70 & 2.84 log CFU/g for T1, T3 % T5, respectively at 40 days of storage. After that, the count of molds and yeasts in the same treatments reached to 4.22, 3.71, 3.25 log CFU/g for T1, T3 % T5, respectively at 50 days of storage as revealed in (**Table 6).** Additionally, the treatments fortified with fig leaves extracts (T4 & T6) free from molds and yeasts count during storage for 50 days. This results due to the preservative action of fig leaves extract, which it's rich with flavonoid and alkaloids compounds that have antimicrobial action against spoilage and pathogenic organisms [45, 61, 62, 63]. But in the T2 was indicated little molds and yeasts count reached 2.00 log CFU/g at 50 days of storage even though the presence of fig leave extract. These may due to the activity of free cells of *Lb. helveticus* which produces faster acidity that encourages the growth of molds and yeasts [47].

3.10. Color changes in functional cheese sauce.

Table 7 demonstrated that the white and yellow color of the functional cheese sauce decreased significantly in the treatments which content fig leaves extract in free or capsules form T2, T4, and T6 compared with other treatments without addition fig leaves extract T1, T3, and T5, whereas the white color was increased at end of storage period than fresh treatments. Furthermore, green color took the opposite way between treatments. The green color of cheese sauce increased significantly in the treatments in which content fig leaves extract T2, T4, and T6 equated with other treatments. Also, the green color was increased at the end of the storage period as well as white and yellow color which took the same approach. The results are in agreement with Mohamed, et al. [19] who observed similar trends. The reason for decreased white and yellow color as well increased the green color of the cheeses content plant leaves extract may be owing to the green color of the extract present in this product.

3.11. Sensory properties of functional cheese sauce during cold storage

Sensory properties of functional cheese sauce fresh and after 50 days of cold storage are shown in **Table 8**. The panelists found that outer appearance scores gradually decreased significantly during the storage period in all cheese treatments. The treatments contains microcapsules *Lb. helveticus* strain using alginate and skim milk without extract (T3 & T5) took higher surface appearance scores than other treatments after 30 days of storage, and this could be related to the green color of these treatments. Furthermore, aroma & flavor scores increased significantly during the storage period and gotten this descending order of flavor acceptability between treatments T4 > T6 > T3 > T5 > T2 > T1 after 30 days of storage.

Moreover, body& texture scores decreased significantly during storage period but took the same previous arrangement of acceptability among different treatments after 30 days of storage. Generally, functional cheese sauce treatments containing microcapsules loaded with *Lb. helveticus* using alginate T4 achieved the highest total score (sum of sensory properties) than other treatments, when fresh and until 50 days of storage. Whereas the lowest total score was observed in control samples (T1) after 30 days of storage.

4. Conclusions

The present study demonstrates that the fig leaves extract have antimicrobial activity against different foodborne pathogens, due to its content of total phenol and antioxidant activity. Also, the microcapsules loaded with fig leaves extract and probiotic strains used in the manufacturing of functional cheese sauce have an extra quality of the final product for 50 days. Physiochemical properties and *Lb. helveticus* count in cheese sauce enhancement during storage and the more improvement detected in treatments with microcapsules. Furthermore, the melting properties in treatments with microcapsules were decreased. Functional cheese sauce containing microcapsules using alginate achieved the highest total score in sensory properties than other treatments. Additionally, the treatments fortified with fig leaves extracts free from mold and yeasts counts during storage for 50 days. Therefore, fig leaves extract with probiotic strains can be supplemented in the functional cheese sauce to add nutritional value and prolong the shelf life.

Table 4. Melting changes of Cheese sauce fresh and during 50 days of cold storage

Samples	Storage Period (days)					
	Fresh	10	20	30	40	50
T1	21.6^{Ad}	25^{Bc}	29.2^{Cb}	32^{Ca}	-	-
T2	20.00^{Bc}	31.8^{Ab}	38^{Aa}	39^{Ba}	-	-
T3	18^{Cc}	20^{Cb}	20.8^{Db}	22.2^{Da}	-	-
T4	13.5^{Dc}	15.3^{Db}	15.8^{Fb}	16^{Fb}	20.2^{Ba}	20.5^{Ba}
T5	17^{Cd}	31.6^{Ac}	34.2^{Bb}	44^{Aa}	-	-
T6	14.5^{Df}	14.7^{De}	17.5^{Ed}	19.7^{Ec}	20.4^{Ab}	21.5^{Aa}

See footnote Table 3

Table 5. Oil separation index (OSI) changes of Cheese sauce fresh and during 50 days of cold storage

Samples	Storage Period (days)					
	Fresh	10	20	30	40	50
T1	0.39^{Cb}	0.40^{Eb}	0.50^{Ea}	0.50^{Fa}	-	-
T2	0.23^{Ec}	0.61^{Cb}	0.61^{Cb}	0.67^{Da}	-	-
T3	0.71^{Ac}	0.71^{Bc}	0.75^{Bb}	0.79^{Ca}	-	-
T4	0.36^{Df}	0.60^{Ce}	0.74^{Bd}	0.84^{Bc}	0.88^{Bb}	1.43^{Ba}
T5	0.40^{Cc}	0.44^{Db}	$0.52^{D}a$	0.53^{Ea}	-	-
T6	0.66^{Be}	0.80^{Ad}	0.80^{Ad}	0.86^{Ac}	1.08^{Ab}	1.49^{Aa}

See footnote Table 3

Table 6. Viability of *Lb. helveticus,* molds, and yeasts (Log CFU/g) in functional cheese sauce treatments during cold storage

Treatments	Storage period (Days)					
	Lb. helveticus					
	Fresh	10	20	30	40	50
T1	9.00^{Ab}	8.56^{Bc}	7.32^{Cd}	6.55^{Dd}	-*	-*
T2	9.09^{Ab}	8.80^{Ab}	8.23^{Bc}	7.67^{Bc}	6.76^{Cc}	-*
T3	9.08^{Ab}	9.09^{Aa}	8.91^{Ab}	8.56^{Bb}	-*	-*
T4	9.06^{Bb}	9.16^{Ba}	9.34^{Aa}	9.20^{ABa}	8.48^{Cab}	7.90^{Db}
T5	9.05^{Ab}	9.07^{Aa}	9.02^{Ab}	8.77^{Bb}	-*	-*
T6	9.25^{Aa}	9.11^{Ba}	9.08^{Bb}	8.99^{Bab}	8.74^{Ca}	8.53^{Da}
	Molds and yeasts					
T1	N.D	N.D	N.D	N.D	3.00	4.22
T2	N.D	N.D	N.D	N.D	N.D	2.00
T3	N.D	N.D	N.D	N.D	2.70	3.71
T4	N.D	N.D	N.D	N.D	N.D	N.D
T5	N.D	N.D	N.D	N.D	2.84	3.25
T6	N.D	N.D	N.D	N.D	N.D	N.D

-* These treatments became moldy. See footnote Table 3.
N.D: Not detectable any molds and yeasts count

Table 7. Color changes of functional cheese sauce fresh and during cold storage for 50 days

Sample	Storage (days)	L*	a*	b*
T1	Fresh	46.14^{Ab}	-0.19^{Aa}	21.34^{Ab}
T2		24.69^{Fb}	-1.55^{Fa}	11.42^{Fb}
T3		39.39^{Bb}	-1.04^{Ba}	17.33^{Bb}
T4		33.68^{Ec}	-1.29^{Ea}	11.80^{Ec}
T5		36.58^{Cb}	-1.23^{Cb}	15.53^{Cb}
T6		34.52^{Dc}	-1.25^{Da}	12.56^{Dc}
T1	30	54.04^{Aa}	-0.78^{Ab}	22.49^{Aa}
T2		28.81^{Fa}	-2.00^{Bb}	13.22^{Fa}
T3		45.23^{Ba}	-1.07^{Ab}	19.38^{Ba}
T4		36.40^{Eb}	-1.40^{ABb}	13.32^{Eb}
T5		41.84^{Ca}	-1.35^{ABc}	19.21^{Ca}
T6		40.55^{Db}	-1.39^{ABb}	14.73^{Db}
T1	50	-	-	-
T2		-	-	-
T3		-	-	-
T4		39.62^{Ba}	-1.49^{Aa}	14.79^{Ba}
T5		-	-	-
T6		45.83^{Aa}	-1.42^{Ba}	15.27^{Aa}

See footnote Table 3. L* value represents darkness from black (0) to white (100). a* value represents color ranging from red (+) to green (−). b* value represents yellow (+) to blue.

Table 8. Sensory properties of cheese sauce fresh and during cold storage for 50 days

Storage Period(days)	Character assessed	T1	T2	T3	T4	T5	T6
Fresh	O.A	18^{ABa}	17^{Ba}	19^{Aa}	17^{Ba}	19^{Aa}	17^{Ba}
	B&T	33^{Ca}	36^{Ba}	37^{ABa}	38^{Aa}	36^{Ba}	37^{ABa}
	A&F	30^{Cc}	33^{Bc}	34^{Bc}	35^{Ac}	34^{Bb}	34^{Bd}
	Total	81^{Da}	86^{Ca}	90^{Aa}	90^{Aa}	89^{ABa}	88^{Ba}
10	O.A	18^{ABa}	17^{Ba}	19^{Aa}	17^{Ba}	19^{Aa}	17^{Ba}
	B&T	31^{Cb}	34^{BCb}	36^{ABab}	37^{Aab}	35^{Ba}	36^{ABa}
	A&F	32^{Bb}	34^{Abc}	35^{Abc}	35^{Ac}	35^{Aab}	35^{Acd}
	Total	81^{Da}	85^{Cab}	90^{Aa}	89^{ABab}	89^{ABa}	88^{Ba}
20	O.A	16^{Cb}	16^{Cab}	19^{Aa}	17^{BCa}	18^{ABab}	17^{BCa}
	B&T	30^{Db}	33^{Cb}	35^{ABb}	36^{Abc}	35^{ABa}	34^{BCa}
	A&F	34^{Ca}	35^{BCab}	36^{ABab}	37^{Ab}	35^{BCab}	36^{ABbc}
	Total	80^{Dab}	84^{Cb}	90^{Aa}	90^{Aa}	88^{Bab}	87^{BCab}
30	O.A	15^{Cb}	15^{Cb}	18^{Aa}	16^{BCab}	17^{ABb}	16^{BCab}
	B&T	28^{Dc}	31^{Cc}	32^{BCc}	35^{Acd}	32^{BCb}	33^{Ba}
	A&F	35^{Ca}	36^{BCa}	37^{ABa}	38^{Aab}	36^{BCa}	37^{ABb}
	Total	78^{Eb}	82^{Dc}	87^{Bb}	89^{Aab}	85^{Cb}	86^{BCb}
40	O.A	--	--	--	16^{Aab}	--	16^{Aab}
	B&T	--	--	--	34^{Ad}	--	33^{Aa}
	A&F	--	--	--	39^{Aa}	--	39^{Aa}
	Total	--	--	--	89^{Aab}	--	88^{ABa}
50	O.A	--	--	--	15^{Ab}	--	15^{Ab}
	B&T	--	--	--	34^{Ad}	--	32^{Bb}
	A&F	--	--	--	39^{Aa}	--	39^{Aa}
	Total				88^{Ab}		86^{Bb}

See footnote Table 3

O.A: Outer appearance (20), B&T: Body& Texture (40), A&F: Aroma& Flavour (40)

Conflicts of interest

In accordance with our policy on Conflict of interest please ensure that a conflicts of interest statement is included in your manuscript here. Please note that this statement is required for all submitted manuscripts. If no conflicts exist, please state that "There are no conflicts to declare".

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