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ARTICLE

Production of Bone Broth Powder with Spray Drying Using Three Different Carrier Agents

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Abstract The aim of this study is to determine the some physicochemical (proximate composition, pH and a_w values) and reconstitution (wettability, dispersibility and solubility index (SI)) properties of spray-dried bone broth powder (BBP) along with the effects of the addition of different carrier agents. Subsequently, the powdered products were stored to determine the storage stability (pH, lipid oxidation, color, browning index) for 3 mon at –18℃. For this purpose, firstly marrow-containing bones (*Os femur* and *Os humerus*) have been boiled to get the bone broth. Three different emulsions were respectively prepared (1) 20% maltodextrin (MD) added BBP, (2) 20% whey powder isolate (WPI) added BBP and (3) 10% MD and 10% WPI added BBP and the emulsions were dried using a spray-dryer with 185℃ inlet and 95℃ outlet temperature. The proximate composition and reconstitution properties of BBP were found statistically different ($p<0.05$) depending on the use of different carrier agents. MD added BBP showed uniform and smooth morphology. The color, browning index, thiobarbituric acid reactive substances (TBARS) and pH values of BBP significantly changed ($p<0.05$) during storage. In conclusion, both the results of physicochemical and storage period analyses showed that the most suitable encapsulation material in the production of the bone broth powder is MD.

Keywords bone broth, encapsulation, spray-dryer

Introduction

Bones are the essential elements of skeleton system of mammals. The organic matrixes of the bones are mainly consist of type-1 collagen and moreover this matrix contains polianionic molecules such as proteoglucan, sialoproteins and fosfoproteins as well (Franzen and Heinegardi, 1984). Also, the bones are one of the most important by products of slaughtering animal carcasses that can be used in both animal and human nutrition. Especially, marrow-containing crushed the bones (*Os femur* and *Os humerus*) are consumed after they boiled and separated from the solid parts, finally the obtained liquid used as the bone broth. Since the bone broth is rich in collagen, calcium, phosporus, hyaluronic acid and chondroitin sulfate. It is known as wound healer, skin and connective tissue regenerator in public health for many years (Aljumaily, 2011).

As it spotlighted beneficial effects on health, the consumption of the bone broth has gradually increased during the recent years. Also, the bone broth can be used as a natural flavor enhancer instead of synthetic ones like monosodium glutamate in foods. For example, it has been used to increase palatability of soups, pilaus and pot dishes. Due to the fact that production of conventional the bone broth has many difficulties such as laborious and time-consuming, it is not produced industrially. However, it has been used by boiling of marrow-containing the bones in small enterprises (restaurants, cafeterias, etc.) and household-scale.

As it's known, the bone broth is a liquid food and occupies large volumes for storing and transportation and also it has limited shelf life due to its chemical composition. Therefore, it is necessary to increase the storage stability of such liquid foods with appropriate preservation methods. Drying is one of the most ideal preservation methods which would practice in liquid foods. With the help of the drying, larger volumes can be substantially decreased and shelf life of final dried products can be extended (Khawas et al., 2014). Although there are several types of drying techniques such as drum, freeze and microwave-vacuum drying, spray drying is an efficient drying method used to transform liquids into powdered product by spraying it continuously into a hot drying medium (Kurozawa et al., 2009). Especially, compared to freeze-drying, the cost of spray-drying method is seen to be more cheaper (Desobry et al., 1997). Moreover, due to their high protein and fat content the bone broth cannot pulverized in spray-dryer without carrier agents alone, it has necessary been used encapsulating with carrier agents. Carbohydrates such as maltodextrin (MD), gums, pectin, starches and corn syrup are usually used as carrier agents in spray drying method. With the exception of MDs with dextrose equivalence between 10 and 20, carbohydrate based on wall materials showed poor interfacial properties and must be chemically modified in order to improve their surface activity. In contrast, proteins have an amphiphilic character which shows excellent emulsification properties and accordingly allows to encapsulate hydrophobic core materials. Moreover, protein compounds such as sodium caseinate, soy protein isolate, and whey protein concentrates and isolates, could also be expected to have good microencapsulating properties. MDs, whey protein and mixture of them isolates are low cost and very useful for spray drying process on food materials. So, encapsulation is an important step in the production of the bone broth powder. Encapsulation is defined as a process in which tiny particles or droplets are surrounded by coating or embedded in a homogeneous or heterogeneous matrix and give small capsules with many properties (Gharsallaoui et al., 2007). Carrier agents provided extra protection against oxygen, water and light which leads to oxidation reactions (Turchiuli et al., 2005). The whey protein isolate, the MD and mixtures of them have been utilized as carrier agents for a good microencapsulation in food industry. The whey protein isolates are by-product of the dairy industry and have high protein content thus, they show good emulsification, gelation and film formation properties in encapsulation process. The MD is an important product obtained from starches and is mainly used in materials that are difficult to dry (Kurozawa et al., 2009). It has been reported that the use of the whey protein isolates in combination with the MD during the encapsulation process provides a nearly smooth surface in the final powdered food particles. This smoothness is important for the functionality of the core material (Sanlıdere-Aloglu and Oner, 2010).

The aim of this study, was to evaluate the effect of the MD, the whey protein isolate (WPI) and mixture of them (MD-WPI) on the physicochemical properties (chemical composition, water activity, particle size, morphology, reconstitution properties) of a spray dried the bone broth powder (BBP). Besides, some quality parameters (pH, color, thiobarbituric acid reactive substances (TBARS) and browning index) of the powdered the product was determined during storage up to 3 mon.

Materials and Methods

Materials

In this study, marrow-containing beef bones (*Os femur* and *Os humerus*) were used to obtain the bone broth as a raw material and they were purchased from local a butcher. The whey protein isolate (moisture, protein, fat and ash content of 3.75, 93.4, 0.57, and 1.82% respectively) and the MD (<20 DE) as carrier agents were purchased from Kavi Food Co. (Hardline Nutrition, Istanbul, Turkey). The WPI and MD were used as received.

Methods

Preparation of the bone broth emulsions

The bone broth was prepared according to the method described by Anonymous (2001). The bones were cut in appropriate size (20–25 cm) with a bone saw. The bones were kept at 30℃–40℃ water for 0.5–1 h to remove any scum that has risen to the top and then scummy water discarded. Subsequently, the bones treated with clean water (1:1, w/v, bone: water) and were boiled again to obtain a high yielded extract for 5–6 h in a pressure cooker. After the boiling process, the bones were discarded and resulting broth was used for further preparation of the bone broth emulsions. The bone broth was left to cool down to 4℃ and excessive fat which is deposited on the surface were removed manually.

Spray-drying

Before the spray-drying process, carrier agents -MD (20%), WPI (20%) and MD-WPI mixture (10+10%) was added directly to the bone broth under magnetic stirring, until complete dissolution. The feed solution having 25% dry matter based on the bone broth.

The drying process was performed in a laboratory scale spray dryer (B290 model, Büchi, Switzerland). The spray dryer was operated with co-current air and solution flow. The nozzle used in the spray drier was a two-fluid self-cleaning nozzle with an orifice of 0.7 mm in diameter. The dimensions of equipment were (W×H×D) 65×110×70 cm respectively and vertically orientated cylinder, with a conical base. The operating conditions of the spray-dryer were given at Table 1.

Proximate composition, pH values, water activity, wettability, dispersibility, solubility index (SI), were performed in the BBP and also pH, TBARS, non-enzymatic browning index (BI) and color (CIE L*, a* and b*) values were measured during storage (3 mon) at –18℃.

Analyses

Proximate composition analyses

The proximate composition (moisture, protein and ash) of the BBP was measured according to AOAC (2006) and fat content carried out according to Flynn and Bramblett (1975).

Table 1. Operating conditions of the spray-dryer

Parameters	Conditions
Inlet air temperature	185℃
Outlet air temperature	95℃
Percentage of aspiration	90%
Percentage of pumping	84%
Feed temperature	50℃

Water activity (a_w)

The water activity of the spray-dried BBP was determined by using the water activity measurement probe (Testo-AG 400, Germany). Approximately, 3–4 g sample is placed to quickly chamber which made from stainless steel and the value of water activity from the indicator of the device directly read and recorded.

Instrumental measurement of color and pH

The color values of samples were performed with colorimeter (Hunterlab Miniscan XE Plus, USA). CIE L* (lightness), a* (redness) and b* (yellowness) values were measured on the outer surface of powder. The pH of the BBP was measured with a digital pH meter (Crison Basic 20, Spain).

Particle morphology

The particle morphology of the BBP samples which dried in incubator at 60℃ were evaluated by a field emission scanning electron microscopy of Pamukkale University Electron Microscopy Unit (FESEM-Carl Zeiss, Supra 40 VP, Germany) at an accelerating voltage of 15 kV. The powders were attached to FESEM stubs using double adhesive tape, coated with gold under vacuum.

Reconstitution properties of powders

The reconstitution properties (wettability, dispersibility and SI) of the powders were determined according to Jinapong et al. (2008).

For the wettability analyses, 0.075 g of powder is weighed and then was passed through from wire mesh screen at a certain height. Afterwards, the powder spattered into 100 mL of distilled water at 35℃. The moment of wetting of the samples is observed and the wetting time by recording the wettability is determined as seconds. Finally, the time was recorded for the powder to become completely wetted (visually assessed as when all the powder particles penetrated the surface of the water).

For the dispersibility analyses, 1 g of powder is added into 100 mL of distilled water and mixture is stirred for 30 s. Following, the mixture was passed through from a sieve (500 μm). The sieved slurry is transferred to a weighed and dried aluminum pan and dry matter analysis is performed in the samples.

$$\text{Dispersibility} = [(mw + mp) \times (M1\% / 100)] / [mp \times (Mp\% / 100)] \times 100$$

mw: weight of distilled water.

mp: weight of the bone broth powder.

M1: dry matter of mixture after ıt has been passed through the sieve.

Mp: dry matter of the bone broth powder.

To measurement of the SI, 1 g of powder was placed into a test tube with 5 mL of 5% sodium chloride solution (m/v). The solution was shaken and mixed with a vortex mixer. The refractive index values (RI) of the dispersed sample and sodium chloride solution were measured by using a refractometer. The SI values were calculated by using Haenni values and SI.

$$\text{Haenni values} = y = RI_{sample} - RI_{NaCl} \times 1{,}000$$

$$\text{Solubility Index (SI)} = [\text{Log}(y) - 0.445] / 0.01$$

Lipid oxidation

TBARS analysis was performed with slight modifications according to Witte et al. (1970), to detect final products of lipid oxidation. Five g of the BBP was homogenized with an ultraturrax in 50 mL 20% trichloroacetic acid and followed by filtration using Whatman no. 1 filter paper. Then, 5 mL filtrate was taken in a screw cap tube followed by the addition of thiobarbituric acid (5 mL, 0.02 M). The tubes were left to develop a yellow color in boiling water bath (70℃) for 30 min and then were left to cool down. At the end of the period, yellow color formation was observed in the test tubes, so the readings were performed at 450 nm (Carry 50 Scan UV, USA). 1, 1, 3, 3, tetraethoxypropane was used as the standard for TBARS assay. TBARS numbers were calculated as mg of malonaldehyde per kilogram of BBP (mg MA/kg BBP).

Browning index (BI)

The BI values of the BBP samples were measured by an enzymatic digestion method (Palombo et al., 1984). The method was based on pronase proteolysis that releases the brown pigments. The pronase solution used in the analysis is prepared by mixing 10 mg pronase with 1 mL Tris buffer (pH:7). 1 g of the BBP placed into a test tube and then 5 mL of distilled water added to the test tube at 50℃ and the powder were dissolved with a tube vortex. Then, 1.5 mL of the dissolved sample was transferred to a new test tube and 0.4 mL of the pronase solution added to the tube and the solution was stirred manually following and was incubated for 120 min in a water bath at 45℃. After the incubation, the test tubes are quickly cooled with iced water and 150 μL of TCA (100%) is added to the samples. Then the tubes are centrifuged at 1,860×g for 20 min at 4℃ in a centrifuge (Nuve NF 800, Turkey). At the end of the centrifugation, the supernatant is collected, and the absorbance of the samples were read at 420 (A_{420}) and 550 (A_{550}) nm by using a spectrophotometer (Shimadzu UV-1601, Japan) and the optical density (OD) of the samples were calculated as follows;

$$OD = A_{420} - A_{550}$$

BI values of the BBP were calculated as dry matter (OD/g). The increasing value of BI indicates that the degree of non-enzymatic browning in the samples.

Statistical analysis

Measurements on each of powder samples were replicated three times and all parameters were measured in duplicate (n=6). A one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were performed to analyze in order to evaluate effects on the treatments and the storage periods by SPSS for Windows (SPSS version 15.0 for Windows).

Results and Discussion

The proximate composition, pH and aw values of the BBP (moisture, protein, fat and ash) were given Table 2.

Analyzing Table 2, the moisture content of the samples was ranged from 6.73% to 7.69% (wet basis) and the results were significantly different ($p<0.05$). To the best of our knowledge, the production of the BBP has not been previously reported thus, other powdered products tried to cite in this study. In our study, the moisture content of the BBPs exceed upon 5% and the main reason why it is considered that caused by the dry matter concentration of the feed solution (25%) and the inlet dryer temperature (185℃).

Table 2. The proximate composition, pH and aw values of the bone broth powder

Samples	Moisture content (%)	Protein (%)	Fat (%)	Ash (%)	pH	a_w
1	6.73[c]	6.19[c]	6.39[c]	0.37[c]	6.64[b]	0.16[b]
2	7.69[a]	11.81[a]	13.98[a]	1.66[a]	6.83[a]	0.20[a]
3	6.94[b]	7.86[b]	7.48[b]	1.00[b]	6.73[ab]	0.17[ab]
SEM	0.29	0.34	0.25	0.06	0.03	0.01

SEM, Standard error of mean (n=6).
[a–c] Different letters within the same column represent significant differences ($p<0.05$).
1, 20% MD added BBP; 2, 20% whey powder added BBP; 3, 10% MD and 10% whey powder added BBP.
BBP, bone broth powder; MD, maltodextrin.

In relation to this issue, it is observed that the moisture content of spray dried chicken meat protein hydrolysates is seen to be below 2% in a study. The authors stated that the main reason why the moisture content remains at such low levels attributed to the concentration of the carrier agent used and the inlet temperature of the in the feed solution itself (Kurozowa et al., 2009). In another study, the moisture content of the cheese powder which produced by using the spray drier is about 2% (Erbay et al., 2015).

The amount of protein among the BBPs were significantly different ($p<0.05$) and the highest protein content was found in sample 2 (11.81%) and it is ascribed to the protein derive from the WPI (Table 2). In a similar study, Kurozawa et al. (2009) found as 7.05% the protein content of spray dried chicken meat hydrolysate.

It is reported that encapsulation is a very important process in the drying of the high fat containing foods (Pauletti and Amestoy, 1999). Also, it is tried to produce BBP without carrier agents in this study but it is not succeed. It is consider that the fat which found in the structure of the bone broth is adhere to the hot glass surface of spray-dryer and it is liquefied and desired final powder product cannot be obtained under this condition. Accordingly, it is seem to be the use of carrier agents is essential in the production of the BBP. By the use of carrier agents, the final fat content of the BBPs were found significantly different in this study ($p<0.05$) (Table 2).

The ash content of all samples were statistically found different ($p<0.05$) (Table 2). The reason for the differences between the results might be owing to the different carrier agents used in this study. The highest ash content was determined in sample 2 (1.66%) and the lowest ash content was observed in sample 1 (0.37%). Caliskan and Dirim (2016) determined that ash content of sumac extract powders varied from 2.46% to 4.22% that depend on high total soluble solid content of sumac extract (12.4 °Bx).

The pH values of samples were ranged between 6.64 and 6.83 ($p<0.05$) (Table 2). WPI added powders showed slightly higher pH values than the MD added ones. In similar studies, different carrier agents affected pH value of the spray dried powders like egg powder (Koc, 2009), cheese powder (Erbay, 2013).

The water activity (a_w) of the BBPs has ranged from 0.16 to 0.20 and treatments are seen to be statistically different ($p<0.05$) (Table 2). WPI added powders have higher water activity values than MD added powders. The powdered foods with low water activity value (below 0.25) were accepted as stable for physical, chemical and microbiological changes (Koc et al., 2010). As for our results, it can be said that spray dried BBPs with low aw values are seem as safe products. In a study by Koc (2009), the aw values of egg powder samples reported that vary from 0.100 to 0.144 and in another study by Koc (2008), the aw values of yogurt powder samples stated that range from 0.096 to 0.166. The aw values of the BBPs have indicated that consistent with the aw values of the powder products in the literature.

The wettability, dispersibility and SI of the BBP was given in Table 3. For powdered foods, the wettability can be defined as the ability of a powder bulk to be penetrated by a liquid due to capillary forces. Some physical properties like particle size, porosity, contact angle and chemical composition which depends on fat, protein and sugar content influences the wettability of powders (Fang et al., 2008). Short wettability times indicate good reconstitution properties and this parameter can vary from product to product. The wettability values of the BBPs ranged from 44.67 to 72.7 s and results were significantly different ($p<0.05$) (Table 3). Uses of the MD which is a carbohydrate origin carrier agent reduced the wettability values in sample 1 whereas uses of the WPI, that is a protein origin carrier agent, increased the wettability values in sample 2 and these results showed that sample 1 indicate the best reconstitution properties among the BBPs.

After the particles are wetted and have sunk, they would immediately start to disperse uniformly as individual particles. To obtain the best reconstitution properties powders need to have good dispersibility, wettability, and optimal agglomeration. Sample 1 and 3 were not statistically different ($p>0.05$) and the dispersibility value of sample 2 has lower than other samples. These results indicated that sample 1 has the best reconstitution properties among the others.

SI is used as indicator in powder product such as egg, milk, rosehip powders and is considered as important a key in determination of the reconstitution quality in food industry (Fang et al., 2008). The SI of the BBPs has observed varied between 71% and 81.6% and the results were significantly different ($p<0.05$) (Table 3). The sample 1 has the highest SI and these results showed that the sample 1 has the best reconstitution properties as it other parameters (wettability and dispersibility).

Jinapong et al. (2008) reported that the wettability values of the soy milk powder vary from 57.4 to 308 s and the dispersibility values of the soy milk powder has ranged from 62.3% to 94%. Erbay (2013) stated that the wettability values of the WPI have ranged from 44.4 to 67.9 s and the dispersibility values of the WPI has varied from 62.76% to 63.45%. However, the dispersibility values of the BBP have ranged from 47.91% to 58.43% in our study. It is considered that the low dispersibility values of the BBP samples ascribed to higher fat content compared to other foods used in the studies. Erbay (2013) determined that the SI of the WPI has varied from 76.95% to 83.06%.

The morphological characteristics of spray-dried the BBPs are shown in Fig. 1. A large number of irregular particles were observed, which is characteristic of powders that are produced by spray drying. The uses of different carrier agents influenced on microparticles morphology of the powdered product. The sample 1 particles which is belong to images showed smooth with no obvious dents particle with shriveled structure (Fig. 1), with no apparent cracks or fissures, which is an advantage, this images showed that capsules have lower permeability to gases, increasing protection and retention of the active material. Similar behaviors were verified for β-caroten (Loksuwan, 2007), flaxseed oil (Carneiro et al., 2013) powders produced by spray drying. The sample 2 particle which is belonging to Fig. 1b images showed spherical and smooth and also some

Table 3. The wettability, dispersibility and solubility index of the bone broth powder

Samples	Wettability (s)	Dispersibility (%)	Solubility İndex (%)
1	44.67^{c}	58.43^{a}	81.16^{a}
2	72.70^{a}	47.91^{b}	71.00^{c}
3	56.02^{b}	58.26^{a}	73.84^{b}
SEM	1.61	0.23	0.33

SEM, Standard error of mean (n=6).
$^{a–c}$ Different letters within the same column represent significant differences ($p<0.05$).
1, 20% MD added BBP; 2, 20% whey powder added BBP; 3, 10% MD and 10% whey powder added BBP.
BBP, bone broth powder; MD, maltodextrin.

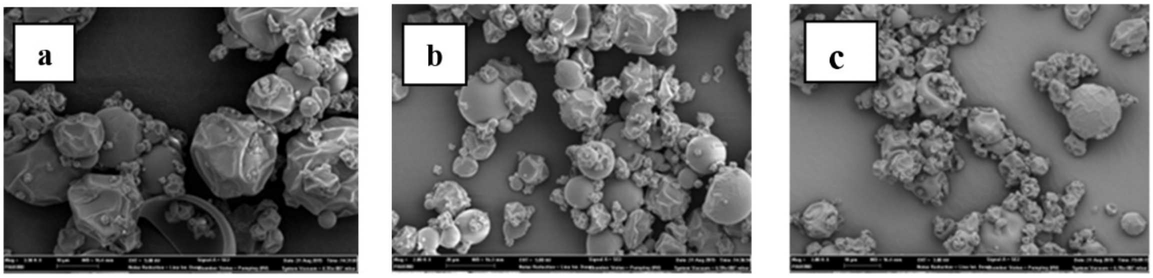

Fig. 1. Spray dried bone broth powders produced using different carrier agents obtained from magnification scanning electron microscopy micrographs (SEM) images of all samples (a) 20% maltodextrin added BBP (bone broth powder), (b) 20% whey powder added BBP and (c) 10% maltodextrin and 10% whey powder added BBP.

shriveled and cracked particles. Similar particle morphology was observed in sample 3. Generally, the spray drying conditions affected the final surface characteristics of droplets such as inlet and outlet temperature, atomization pressure and some emulsion properties like dissolved gases from the emulsion during drying and residual moisture content (Kurozawa et al., 2009). Rosenberg et al. (1985) indicated that the presence of dents had an adverse effect on the flow properties of the powders. Surface imperfections, such as wrinkles, cracks, or collapses, occur when a film slowly forms during atomization according to Re (1998). In encapsulated ingredients smooth spheres are desirable for the stability and solubility of droplets (Osorio et al., 2011).

The pH, TBARS and BI of the BBPs were given in Table 4 during storage. At mon 0 and 2, the uses of different carrier agents were showed significant differences on pH values of the samples ($p<0.05$), though at mon 1 the pH values of sample 2 and 3 were found slightly higher than sample 1 and at mon 3 sample 2 showed the highest pH value. During storage, the pH values of the all samples were gradually increased until the end of mon 1 and a decrement were observed during mon 2 and again the pH values increased rapidly after the second mon ($p<0.05$).

Foods containing unsaturated fatty acids at high concentration are highly susceptible to lipid oxidation. The lipid oxidation causes major changes on quality characteristics of foods such as color, taste, aroma and texture and also leads to the formation of toxic compounds. In generally, to determine the level of lipid oxidation, TBARS analysis is used (Demirkaya, 2013). The TBARS values of samples varied from 0.10–0.26 mg MA/kg BBP and our results remained under threshold value (2.5 mg MA/kg BBP). In this study, from mon 0 to mon 1, sample 1 has showed highest TBARS value (Table 4). At mon 0 and 1, the TBARS value of the sample 2 and 3 were not significantly different ($p>0.05$) although sample 1 were statistically different from the other samples ($p<0.05$). At mon 2 and 3; the sample 3 has the highest TBARS value and sample 1 and 2 were not statistically different ($p>0.05$) and also sample 3 was significantly different from the other samples ($p<0.05$). At the end of the storage, sample 3 has highest the TBARS value. Except sample 1, the TBARS values of the other samples were gradually increased compared to the initial values throughout storage. Dzondo-Gadet et al. (2005) reported that two different encapsulating methods with MD (spray and freeze drying) of safou oil significantly inhibit lipid oxidation effectively compared to non-encapsulated safou oil during storage in terms of TBARS values.

The Maillard reaction is a chemical reaction between amino acids and reducing sugars. It occurs in three major stages and it is depend on factors such as pH, time, temperature, concentration of reactants etc. (Ames, 1998; Yildiz et al., 2010). The reaction is a form of non-enzymatic browning which typically proceeds rapidly from around 140℃ to 165℃. Moreover, the outlet temperature is the most important factor for Maillard reactions in the spray drying process. It is reported that the Maillard reaction slows down with the reduction of the moisture content of the powders and it stops $a_w<0.2$ (Erbay, 2013). Maillard reaction which can have toxicological implications such as the formation of acrylamide causes browning of foods

Table 4. The pH, TBARS and browning index value of the bone broth powder during storage

	Storage times (mon)			
Samples	0	1	2	3
pH				
1	6.64^{cC}	6.76^{bB}	6.57^{cD}	6.91^{bA}
2	6.83^{aC}	6.95^{aB}	6.91^{aB}	7.12^{aA}
3	6.73^{bC}	6.93^{aA}	6.85^{bB}	6.93^{bA}
SEM	0.05	0.04	0.07	0.10
TBARS (mg malonaldehyde/kg product)				
1	0.10^{aC}	0.15^{aB}	0.11^{bC}	0.20^{bA}
2	0.03^{bC}	0.05^{bC}	0.11^{bB}	0.20^{bA}
3	0.05^{bC}	0.07^{bC}	0.19^{aB}	0.26^{aA}
SEM	0.01	0.01	0.04	0.01
Browning index (BI)				
1	0.06^{bD}	0.16^{aC}	0.21^{bB}	0.36^{abA}
2	0.09^{aD}	0.17^{aC}	0.22^{bB}	0.40^{aA}
3	0.09^{aD}	0.16^{aC}	0.28^{aB}	0.34^{bA}
SEM	0.01	0.01	0.01	0.03

SEM, Standard error of mean (n=6).
$^{a–c}$ Different letters within the same column represent significant differences (p<0.05).
$^{A–D}$ Different letters within the same row represent significant differences (p<0.05).
1, 20% MD added BBP; 2, 20% whey powder added BBP; 3, 10% MD and 10% whey powder added BBP.
BBP, bone broth powder; MD, maltodextrin.

(Boekel, 2006). BI is the best indicator of the browning reactions. At the beginning of the storage, although the BI of the sample 2 and 3 were not statistically different (p>0.05), the sample 1 was significantly lower than the others (p<0.05) (Table 4). At mon 1, the samples were not statistically different (p>0.05). At mon 2, the sample 3 were showed higher BI values compared to others and also sample 1 and 2 were found similar (p>0.05). At the end of the storage, the highest BI values were observed in sample 2. After 3 mon storage; the BI values of the spray-dried powders increased approximately 6 times in sample 1, 5 times in sample 2 and 4 times in sample 3 greater than the initial value. At the end of storage, the increment in the BI value is thought that cause decrement in the L* (lightness) value of the powder products (Erbay, 2013). Kilic et al. (1997) stated that the BI increased with increasing temperature and storage time in Cheddar cheese powder. In a study, the BI of both the freeze-dried and spray-dried bayberry powder significantly increased during storage period (50 d) (Cheng et al., 2017).

Color plays very important role in consumption of powdered foods. It gives the consumers the direct visual correlation to physical properties of the powder product such as freshness and taste (Balaban et al., 2005). Generally; the color of powders depend on drying method, temperature, particle size, carrier agent type that is used for encapsulation, etc. (Caparino et al., 2012). Moreover, the CIE L* and a* values in animal origin foods (egg, dairy and bone) show variability depending on the oxidation of lipid and protein fractions (Ozunlu et al., 2018).

The CIE L* values of the BBPs ranged from 72.44 to 77.42 (Table 5). Except mon 3, the sample 1 has the highest CIE L* values each of experimental mon and significantly different from other samples (p<0.05). At mon 1 and 2, though the sample 1 and 3 were found similar (p>0.05), the sample 2 was different from the others. At the end of the storage; although the

Table 5. **The color parameters of the bone broth powder during storage**

	Storage times (mon)			
Samples	0	1	2	3
L* (lightness)				
1	77.42^{aA}	76.66^{aA}	75.45^{aB}	72.44^{bC}
2	75.62^{bA}	74.95^{bB}	73.68^{bC}	73.52^{aC}
3	75.73^{bA}	75.57^{aA}	74.17^{abB}	73.77^{aC}
SEM	0.20	0.13	0.17	0.12
a* (redness)				
1	–1.01^{bA}	–1.36^{bB}	–1.91^{cC}	–2.13^{aD}
2	–0.96^{aA}	–1.31^{aB}	–1.74^{aC}	–2.12^{aD}
3	–1.02^{bA}	–1.38^{bB}	–1.85^{bC}	–2.16^{bD}
SEM	0.01	0.01	0.02	0.01
b* (yellowness)				
1	4.11^{cB}	4.37^{cA}	3.05^{cC}	2.45^{cD}
2	7.61^{aA}	7.18^{aB}	7.12^{aB}	5.60^{aC}
3	6.33^{bA}	5.89^{bB}	5.84^{bB}	4.09^{bC}
SEM	0.10	0.07	0.08	0.09

SEM, Standard error of mean (n=6).
$^{a–c}$ Different letters within the same column represent significant differences (p<0.05).
$^{A–D}$ Different letters within the same row represent significant differences (p<0.05).
1, 20% MD added BBP; 2, 20% whey powder added BBP; 3, 10% MD and 10% whey powder added BBP.
BBP, bone broth powder; MD, maltodextrin.

sample 2 and 3 were found similar (p>0.05), the sample 1 was statistically different from the other samples (p<0.05). Throughout storage, lightness of all samples decreased gradually, and it is considered to realize the formation of secondary oxidation products. As a result of the oxidation analysis; the increase in the TBA value of the sample 1 supports the decrease in CIE L* value up to 3 mon (Demirkaya, 2013). Rodriguez-Hernandez (2005) reported that the addition of different levels commercial MD (18% and 23%) used as carrier agents, decreased the CIE L* values of cactus pear juice (*Opuntia streptacantha*) in spray-drying. Decreasing in CIE L* values correlated with increases in the browning of foods (Chua et al., 2001; Koyuncu et al., 2003). In another study, Caparino et al. (2012) determined that the three different methods (freeze drying, drum drying and spray drying) and the addition of commercial MD (DE:10) used as carrier agents increased the CIE L* values of mango (Philippine 'Carabao' var.) in spray-drying. Abonyi et al. (2002) and Jaya et al. (2006) stated that the CIE L* values of powders of the spray dried was lighter than the other drying process and reported highly effective of the addition of MD which was necessary to reduce the stickiness of the mango in the spray-drying process.

The CIE a* values of the samples ranged from –0.96 to –2.16 during storage (Table 5). Although the sample 1 and 3 did not significantly different (p>0.05) at mon 0 and 1, they were statistically different the other experimental mon (p<0.05). Till the end of mon 1, the greenness values of BBP's were remain relatively low however and then it is rapidly increased. At mon 2, the CIE a* values of all samples were significantly different (p<0.05) and the highest greenness were observed in MD added BBP. At end of the storage, though the sample 1 and 2 were found similar (p>0.05), the sample 3 was found higher from the others (p<0.05). During storage, the CIE a* values of all samples statistically different (p<0.05) and gradually decreased. It is observed that the CIE a* values of the all samples decreased up to 3 mon owing to the brownish/greenish color of the bone broth. It's well known that, with Maillard reactions generally brownish colored Maillard products can be formed in spray dried products and these pigments induced to oxidation reactions enzymatically. Accordingly, the color of powdered products changed substantially during the storage (Palombo et al., 1984). It is showed that the use of different type and amount of carrier agents and drying methods affected the CIE a* values of final powdered products (Que et al., 2008; Wang et al., 2009).

The CIE b* values of the BBPs ranged from 2.45 to 7.61 (Table 5). The sample 2 has highest CIE b* values at all experimental periods. The CIE b* values of the samples were statistically different on all storage mon ($p<0.05$). However, the CIE b* values of the sample 1 showed fluctuating curve throughout storage. From mon 0 to mon 1; although the CIE b* values of the sample 1 increased, from mon 1 to 3, it is decreased. Except sample 1, the CIE b* values of the other samples were significantly different during storage ($p<0.05$).

Caliskan and Dirim (2016) stated that addition of different amounts of MD (DE:10 and 12) the CIE b* values of the spray dried sumac extract powders increased and the CIE b* values of the freeze dried sumac extract powders decreased.

Conclusion

In this study, it was possible to evaluate the performance of three different carrier agents combinations in the bone broth microencapsulation by spray drying. Although authors expected that uses of WPI is an animal origin carrier agent for bone broth production is more suitable than other carrier agents (MD and mixture of them etc.), the results indicated, contrary to expectations. According to results obtained from this study, uses of the MD come into prominence as an appropriate carrier agent for the bone broth production in terms of physicochemical, reconstitution properties (wettability, dispersibility and SI) and scanning electron microscope images. Also, 20% MD added BBP did not show any negative effect in terms of pH, lipid oxidation and browning index throughout the storage. In conclusion, spray drying of the bone broth is fairly an effective method in terms of storage and ease of transport. In industrial scale, the spray drying technique has been successfully used in foods of animal origin such as egg, yogurt and cheese powder so it is consider that the BBP could be produced commercially with spray drying. Consequently, the BBP might be used to enhance palatability of soups, pilaus and pot dishes as a new industrial product in food industry. Exportation of encapsulated BBP could be a prominent income for Turkey because of the bone broth nutritional value.

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