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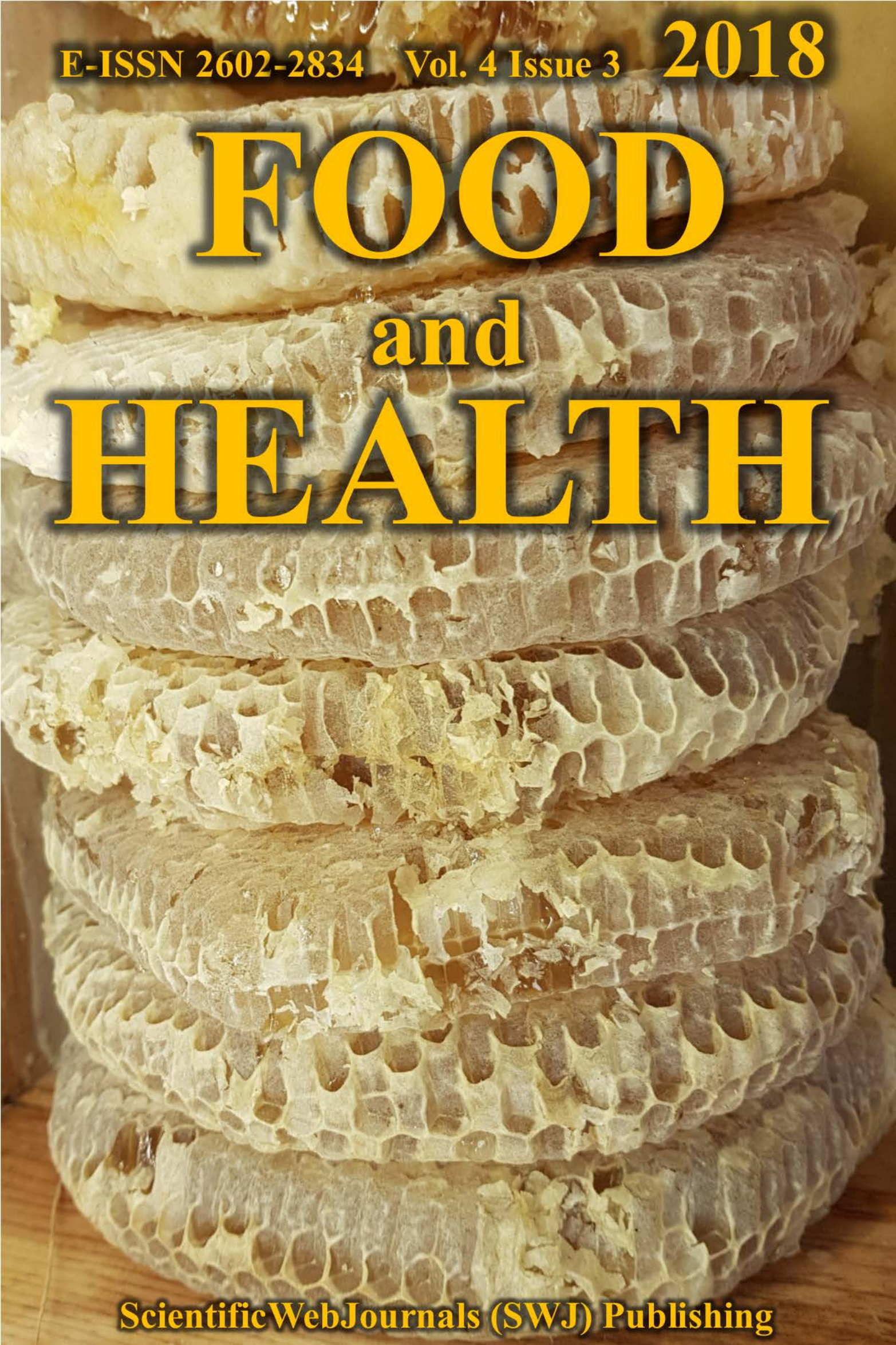
FOOD and HEALTH



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Aims and Scope

"Food and Health" journal will publish peer-reviewed (double blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their team of experts provides editorial excellence, fast publication processes and high visibility for your paper.

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Manuscripts submitted to "Food and Health" journal will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. Our journal will be published quarterly in English or Turkish language. "**Food and Health"** journal will not charge article submission or processing fees.

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THE EFFECT OF SORBATE ON MICROBIOLOGICAL, SENSORY PROPERTIES AND RIPENING PARAMETERS OF KASHAR CHEESE

Ayşen Babacan[1], Salih Özdemir[2]

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[1] Ankara University, Kalecik Vocational School, Kalecik, Ankara, Turkey

[2] Atatürk University, Department of Food Engineering, Faculty of Agriculture, Erzurum, Turkey

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Correspondence:

Salih ÖZDEMİR

E-mail: ozdemirs@atauni.edu.tr

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ABSTRACT

In this study, two different salting methods (dry salting and salted in scalding water) and also application of potassium sorbate to prevent mold growth (in 5% concentration for 2 minutes immersion method) were investigated effects on quality properties of Kashar cheese. Kashar cheese samples were ripened at 4 ±1°C for 5th, 30th, 60th, 90th days and ripening parameters compared during ripening periods. According to the microbiological analysis results, total aerobic meshophylic bacteria (TAMB), lactic acid bacteria (LAB) growth on MRS and M-17 agar and mould counts of Kashar cheeses were found statistically different among all samples ($P<0.01$). The highest mold count (3.58 log cfu/g) was found at control samples salted in scalding water. The lowest count (1.48 log cfu/g) was found at sorbated samples salted as dry. The mould counts of the Kashar cheese samples treated with potassium sorbate were under of standart level (maximum 2 log cfu/g). But, sorbate was not completely prevent mold growth. Coliform group bacteria counts were determined <1 log cfu/g and S. aureus counts were determined <2 log cfu/g at all samples. It was found that acidity values increased in all Kashar cheese samples during the ripening periods. Acidity values of all control groups were higher than that of sorbated samples. The WSN, TCA-TN, PTA-TN and RI values increased in all Kashar cheeses during ripening periods. According to the sensory analysis results, it was determined that there was no adverse effect of sorbate on the sensorial quality of samples during all ripening periods. Panelists preferred the samples salted in scalding water than that of dry salted samples.

Keywords: Kashar cheese, Sorbate, Ripening, Mould, Sensorial analysis

Introduction

Today, most of cheese types have registered trademarks and have protected geographic indications. Kashar cheese is one of the most important cheese types in Turkey and it can also be seen as the most typically stretched curd or pasta-filata cheese (Yangılar and Yıldız, 2016). It is also known as Kashkaval, Kasseri and Caciocavallo in different countries (Güler, 2005). The scalding and kneading stages at Kashar cheese production are the most important stages to form the characteristic properties of the cheese. Kashar cheese has smooth, dry and yellowish rind, homogenous structure without gas holes, its flavor is piquant and slightly salty (Üçüncü, 2004; Yalman et al., 2015).

Food additives are generally added to processed foods for prolong the shelf-life by protecting them from deterioration caused by microorganisms (Mpountoukas et al., 2008). The chemical preservatives widely used at food industry are benzoic and sorbic acids and their salts (sodium benzoate and potassium sorbate) (Tfouni and Toledo, 2002; Gül and Dervişoğlu, 2013). Potassium sorbate (PS) is commonly used as a food preservative because of considered as a "generally recognized as safe" (GRAS) (Famá et al., 2006; Kristo et al., 2008; Türe et al., 2013).

The salting of cheese generally can be made with as dry salting and in scalding water in our country. Cheese salting stage is both consumer demand, as well as charter and other technological process for the realization of the desired qualities of a cheese production standards. The salting of Kashar cheese is an important process for the Kashar cheese quality (Üçüncü, 2010). Generally, cheeses are salted for being of colour and texture and increasing the storage times (Payne and Morison, 1999). Salt in the cheese controls the enzyme activity and ripening (Güven and Karaca, 2001). Generally, dry salting method is used at old Kashar cheese making. There are some disadvantages of dry salting of Kashar cheese. In dry salting, salt is used at more amounts and salting isn't homogen.The other method of salting of Kashar cheese is salting in scalding water. This method provides the homogen salt penetration. In this method, Kashar cheese is salted in hot brine of 75-85°C for 3-5 minute (Say, 2008).

According to Turkish Standard (3272) (TSE, 2006), this cheese is classified as 'fresh Kashar cheese' and 'old or matured Kashar'. Most cheese varieties (is one of Kashar cheese) require a period of ripening (least 90 days) (TSE, 2006) for development of flavour and texture characteristics (McSweeney and Fox, 1997). But, at this ripening period the most important problem is moldy surface of Kashar cheese during ripening. For this reason, the Kashar cheese must be cleaned from moldys before eating. Mold growth on the cheese surface both causes economical loses (cheese lost of approximately 8%) and results healty problems because of mycotoxins producing (Türe et al., 2011). So, Kashar cheese must be controlled the fungal growth by different antimicrobial agents. They can be applied to food surfaces with different methods (dipping, spraying, or brushing). According to the Turkish Food Codex Regulations on Food Additives, potassium sorbate (E 202) is one of the allowed antimicrobial agent to the ripened cheese surface and have not determined any maximum limit (Anonymous, 2013). In a lot of country (ABD, Australia, Finland and Canada) of world are permitted to potassium sorbate at 3000 ppm level. In Turkey, it is permitted to potassium sorbate adding at 1000 ppm levels (Üçüncü, 1980).

Materials and Methods

Material

Raw milk samples used at Kashar cheese making were obtained from by the Research and Application Farm of Atatürk University. Salt and potassium sorbate (Fluka) were used at cheese making process. Commercial microbial liguid rennet (1/15 000 strength) was obtained from Mayasan Company, commercial rock salt used for salting and it was diluted in the sized of 1-2 x 10^{-3} m.

Methods

Kashar cheese samples for using this search were produced Atatürk University Faculty of Agriculture, Department of Food Engineering in the pilot plant.

Kashar cheese samples were made with cow milk. The fat ratio of milk was standardized to 3% with a skim milk addition (Üçüncü, 1980). The procedures of producing the various Kashar cheese samples were:

Cheese 1 (A): The cheese samples were produced with salted in scalding water. The potassium sorbate application was not made the cheese samples.

Cheese 2 (B): The cheese samples were produced with salted in scalding water and treated with potassium sorbate (5%) for 2 minutes.

Cheese 3 (C): The cheese samples were produced with dry salting. The potassium sorbate application was not made the cheese samples.

Cheese 4 (D): The cheese samples were salted as dry and treated with potassium sorbate (5%) for 2 minutes.

Microbiological Analysis

In this research, 10 g Kashar cheese samples were homogenized in 90 mL of a sterile solution (0.85% NaCl) using a stomacher (Lab. Stomacher Blander 400 BA 7021,

Swardmedical). Further decimal dilutions were prepared with the same diluent (Harrigan, 1998). Analyses were carried out using the following procedures:

Total aerobic mesophilic bacteria were enumerated on plate count agar (Merck) following the pour-plate method and with aerobic incubation at 30 ±1°C for 48 h (Messer et al., 1985). Coliform counts were determined by the Violet Red Bile Agar (Oxoid) with plate incubation at 35 ± 2°C for 48 h (Diliello, 1982). LAB counts were determined by MRS-agar (Oxoid) M-17 agar (Gilliand et al., 1984) following the pour-plate method and incubated at 30°C for 48 h. Moulds were enumerated on Potato Dextrose Agar (PDA) (Merck) following the pour-plate method and incubated at 25°C for 5-7 days (Koburger & Marth, 1984). *Staphylococcus aureus* enumeration of samples were made on Baird Parker Agar and incubated at 37°C for 48h. Then, kathalase and cuagulase tests were made on the bacteria colonies (Tatini et al., 1984).

Chemical Analysis Methods

The protein, water soluble nitrogen (WSN), ripening index, pH and titratable acidity (%) were carried out according to the methods by Kurt et al. (2007). For nitrogen analysis solubled in TCA, TCA solution (24%) added to filtrate of 25 mL and sold for 2 hours. Then mix was filtrated and protein analysis was made with filtrate (Polychroniadou et al., 1999). The nitrogen analysis solubled in PTA was made according to Jarret et al. (1982). The 5 mL WSN extract, 3.5 mL 3.95 M H_2S0_4 and 1.5 mL 33.3% PTA solition were mixed. Then, the solution was hold for 12 hours and filtrated with whatman no: 42. Then, nitrogen amount in the solution was found with Kjeldahl method.

Sensory Evaluations

Six panellists experienced in the sensorial evaluation of cheese assessed the cheese samples on 5^{th}, 30^{th}, 60^{th} and 90^{th} days of ripening considering the method of Bodyfelt et al. (1988) and Altuğ (1993). Samples were scored considering five sensorial features e.g., colour, texture, taste and aroma, bitterly, salty and general acceptability ranging from 1 (poor) to 9 (excellent).

Statistical Analysis

All statistical analysis was performed using SPSS Statistical Software, version 17. Mean values with a significant difference were compared by Duncan's multiple range tests. All analyses were performed in duplicate.

Results and Discussion

The some microbiological and chemical anlysis results of raw milk sample were summarized in Table 1.

The logarithmic counts of the microorganisms enumerated throughout ripening time for Kashar cheese samples are presented in Table 2.

Table 1. The chemical and microbiological analysis results of raw milk sample

Microbial groups (log cfu/g)	**Raw milk**
TAMB Counts	7.60
LAB growth on MRS agar	6.84
LAB growth on M-17 agar	7.37
Coliform groups	4.70
Moulds-yeasts	3.48
Chemical Characteristics	
Dry matter (%)	12.66
Fat (%)	4.00
Protein (%)	2.93
Acidity (lactic acid%)	0.18
pH	6.65

Table 2. The some microbiological analysis results of Kashar cheese samples (log cfu/g)

Samples	Ripening Times (days)	Total mesophilic bacteria	Lactobacilli	Streptococci	Moulds
A	5	7.85±0.050^{B,d}	8.13±0.184^{B,b}	7.79±0,050^{B,c}	<1.00^{A,a}
	30	7.67±0.078^{A,ab}	7.31±0.014^{A,a}	7.53±0.085^{A,a}	3.40±0.141^{B,b}
	60	7.67±0.028^{A,b}	7.48±0.035^{A,b}	7.45±0.035^{A,a}	4.75±0.021^{C,d}
	90	7.62±0.000^{A,c}	7.44±0.014^{A,c}	7.49±0.035^{A,b}	5.26±0.106^{D,d}
B	5	7.44±0.021^{A,b}	7.51±0.000^{B,a}	7.54±0.035^{A,b}	<1.00^{A,a}
	30	7.77±0.021^{C,b}	7.43±0.070^{B,b}	7.72±0.021^{B,b}	2.25±1.061^{A,ab}
	60	7.66±0.021^{B,b}	7.31±0.014^{A,a}	7.63±0.057^{AB,b}	<1.00^{A,a}
	90	7.63±0.050^{B,c}	7.47±0.014^{B,c}	7.62±0.021^{AB,c}	2.12±0.170^{A,b}
C	5	7.63±0.042^{B,c}	8.17±0.007^{C,b}	8.14±0.007^{C,d}	<1.00^{A,a}
	30	7.61±0.014^{B,b}	7.31±0.007^{B,a}	7.58±0.085^{B,ab}	<1.00^{A,a}
	60	7.32±0.028^{A,a}	7.28±0.035^{B,a}	7.48±0.000^{B,a}	1.30±0.000^{B,c}
	90	7.28±0.000^{A,a}	7.01±0042^{A,a}	7.26±0.007^{A,a}	3.94±0.007^{C,c}
D	5	7.31±0.000^{A,a}	7.30±0.000^{B,a}	7.10±0.028^{A,a}	<1.00^{A,a}
	30	7.75±0.035B,a	7.57±0.028^{D,c}	7.62±0.021^{C,ab}	2.76±0.106^{C,b}
	60	7.76±0.120^{B,b}	7.50±0007^{C,b}	7.63±0.042^{C,b}	1.00±0.000^{A,b}
	90	7.36±0.000^{A,b}	7.19±0.028^{A,b}	7.49±0.028^{B,b}	1.24±0.000^{B,a}

*Samples showing capital letters (during storage days) and lower letters (between cheeses at the same storage day) do not differ significantly (P>0.05)

The TAMB counts of Kashar cheese samples changed at arround 7.28 log cfu/g and 7.85 log cfu/g (Table 2). The differences of cheese species, salting methods and ripening periods were found significant (P<0.01). The TAMB counts at A and C samples decreased as ripening periods increased. It was found that the TAMB counts of cheese samples salted as dry were lover than that of in scalding water. This state can be sourced from begining of the early coat and anaerobic state at samples dry salted (Johnson and Law, 2010).

The lactobacilli counts at samples salted as dry were lower than that of scalding water (P<0.05). It says that dry salting of Kashar cheese was decreased the lactobacilli counts because of irregular distribution of salt. The streptococci counts were between 7.26 log cfu/g and 8.14 log cfu/g (Table 2). Generally, as ripening periods increased, streptococci counts of samples decreased. At all samples, the coliform counts were under 1.00 log cfu/g. The mould counts of Kashar cheese samples were between <1.00 log cfu/g and 5.26 log cfu/g (Table 2). The highest mould count was found at control samples salted in scalding water, salted as dry and sorbated samples contained the lowest counts. The mould counts of control samples increased as ripening periods increased. Özdemir and Demirci (2006) found that the potassium sorbate treatment to Kashar cheese was decreased the yeast and mould counts of samples. These results of Özdemir and Demirci (2006) have similarities with our yeast and molds counts of samples.

The results of acidity, pH, WSN contents, ripening index values, TCA-SN and PTS-SN values of Kashar cheese samples are given in Figure 1, 2, 3, 4 and 5. The lowest acidity value (0.72%) was found at raw B samples, the highest value (1.04%) was found at A samples ripened for 30 days and C samples ripened for 90 days. From Duncan's test result, as a samples had the highest acidity (0.95%), D samples had the lowest acidity (0.78%). At the first of ripening periods, the awerage acidity of samples were 0.75%, the acidity of samples ripened for 90 days was found as 0.93%. The acidity of cheese is according to lactic acid bacteria counts, lactose amount and ripening state factors (Say, 2008). Researches (Arıtaşı, 1990; Koçak et al., 1996; Sert et al., 2007) found that as the ripening periods increased, the acidity of Kashar cheese samples increased. Özdemir and Demirci (2006) found that the potassium sorbate addition of cheese

was effected the acidity value of cheese samples. We found that the acidity of the samples preserved with potassium sorbate were lower than control samples. The results of Özdemir and Demirci (2006) weren't parallel with our results.

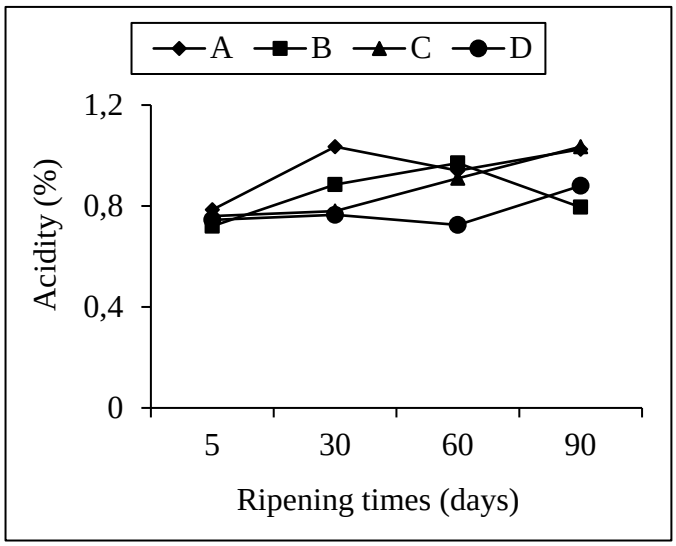

Figure 1. The changes in acidity values of Kashar cheese during ripening: A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

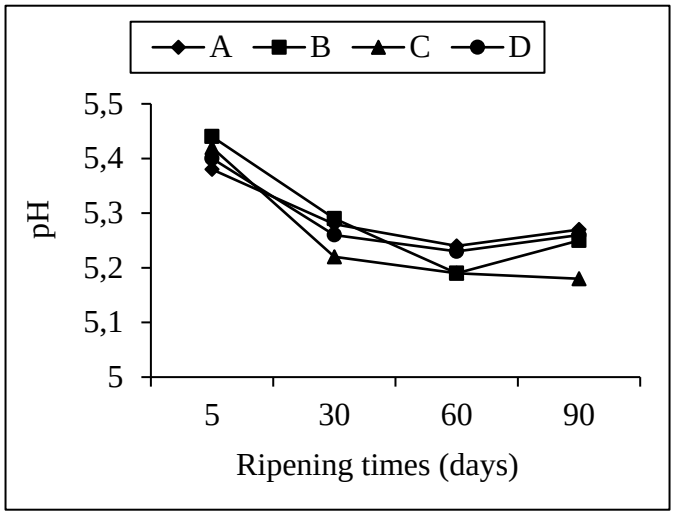

Figure 2. The changes in pH values of Kashar cheeses during ripening: A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

The results of acidity, pH, WSN contents, ripening index values, TCA-SN and PTS-SN values of Kashar cheese samples are given in Figure 1, 2, 3, 4 and 5. The lowest acidity value (0.72%) was found at raw B samples, the highest value (1.04%) was found at A samples ripened for 30 days and C samples ripened for 90 days. From Duncan's test result, as a samples had the highest acidity (0.95%), D samples had the lowest acidity (0.78%). At the first of ripening periods, the awerage acidity of samples were 0.75%, the acidity of samples ripened for 90 days was found as 0.93%. The acidity of cheese is according to lactic acid bacteria counts, lactose amount and ripening state factors (Say, 2008). Researches (Arıtaşı, 1990; Koçak et al., 1996; Sert et al., 2007) found that as the ripening periods increased, the acidity of Kashar cheese samples increased. Özdemir and Demirci (2006) found that the potassium sorbate addition of cheese was effected the acidity value of cheese samples. We found that the acidity of the samples preserved with potassium sorbate were lower than control samples. The results of Özdemir and Demirci (2006) weren't parallel with our results.

The ripening index of cheese is according to dfferent factors as waste renning amoung, packaging material, and salt and water ratio of cheese and storage stages. The ripening degree of cheese samples changed at between 4.13% and 14.37%. At Duncan test results, it was found that D sample had the highest ripening degree. Usually, ripening index of the samples added potassium sorbate had higher than that of unsorbatted samples.We can say that the sorbate addition to Kashar cheese was wasn't show no adverse effect on ripening of Kashar cheese. As the ripening periods increased, the ripening degree increased too. The results was parallel with Say (2008).

The soluble nitrogen in TCA is consist of peptides with short chain and amino acids (Fox, 1989). It was found that the lowest and highest soluble nitrogen in TCA ratio were found 0.18% and 0.29%, respectively. The TCA-SN ratio of D samples were higher than that of other samples. The TCA soluble protein ratio in cheese samplesis according to starter culture and waste rennet amount (Mc Sweeney and Fox, 1997). As the ripening periods increased, TCA-SN ratio increased too. Similar results were found by Yılmaz and Dağdemir (2012) too.Yaşar (2007) and Say (2008) found the similar reseults with ours. Lau et al. (1991) found that the TCA-SN ratio of Cheddar cheese samples increased as the ripening times increased.

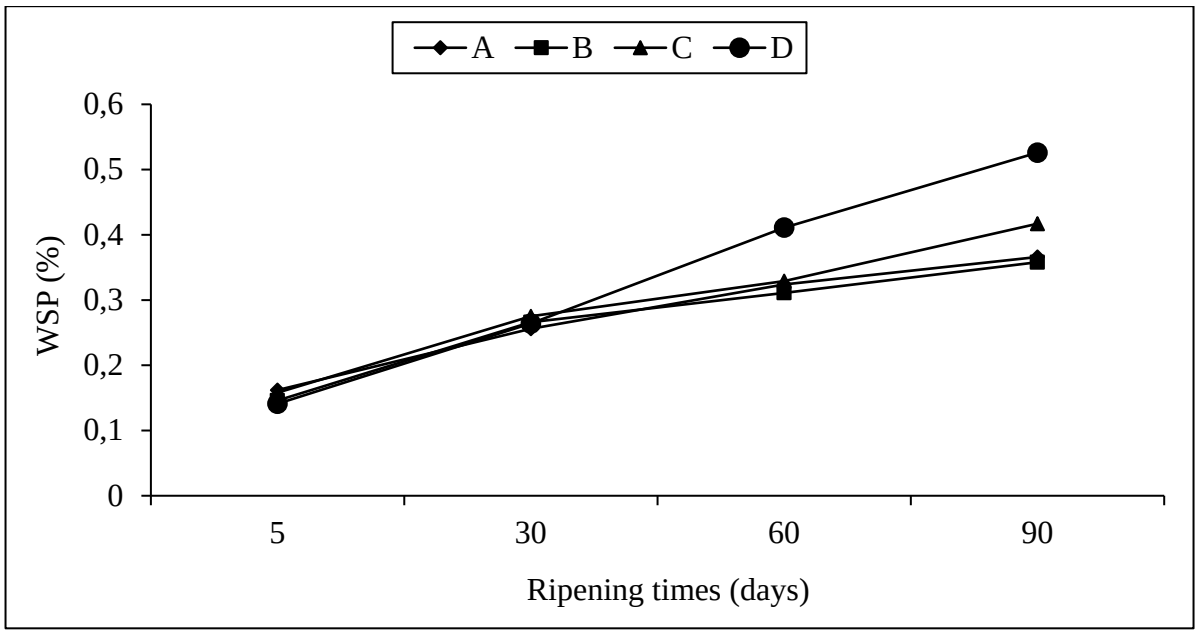

Figure 3. The changes in WSN contents of Kashar cheeses during ripening A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

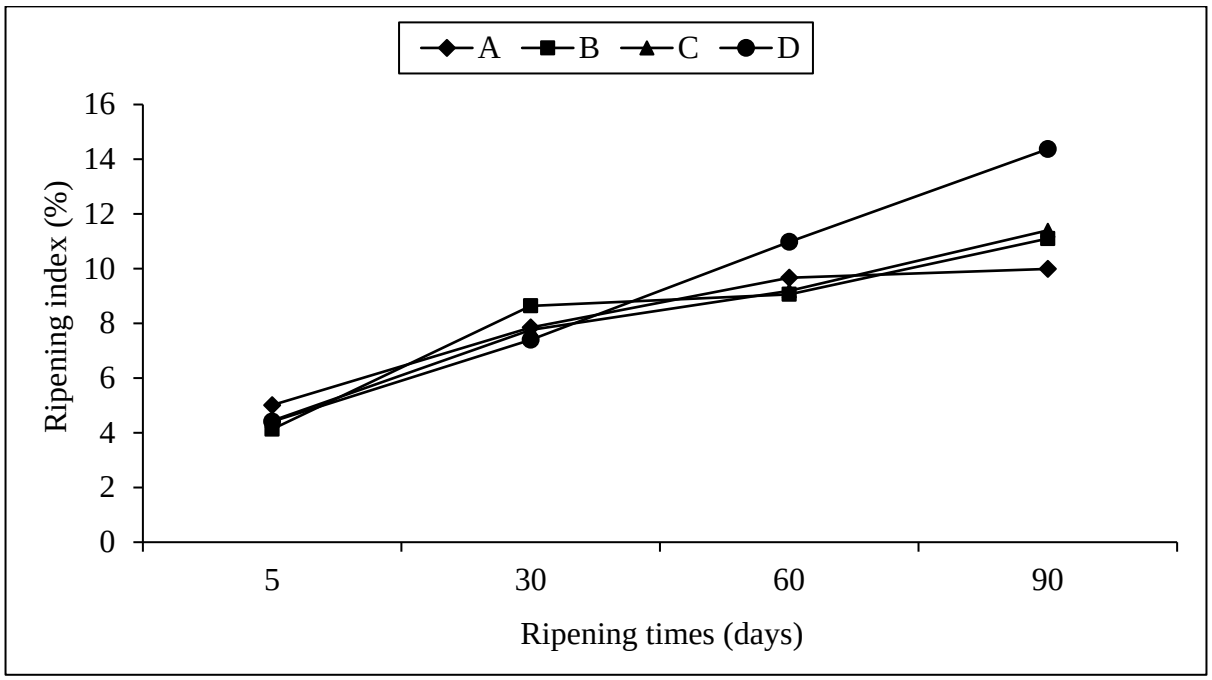

Figure 4. The changes in ripening index values of Kashar cheeses during ripening A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

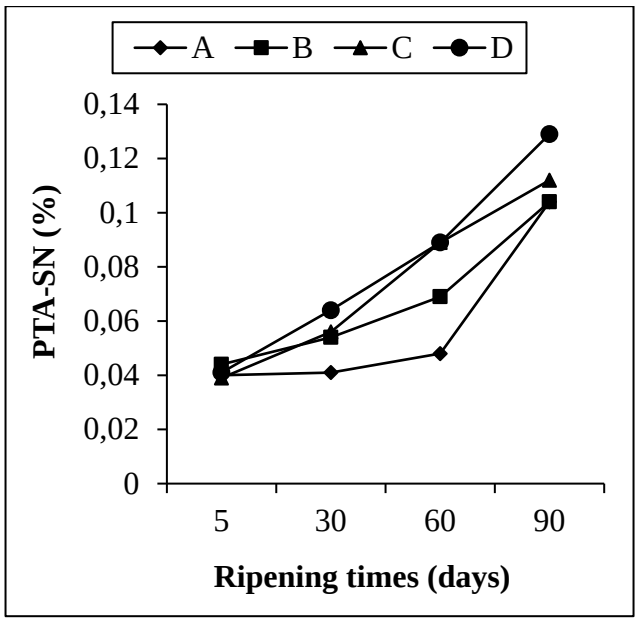

Figure 5. The changes in TCA-SN values of Kashar cheeses during ripening A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

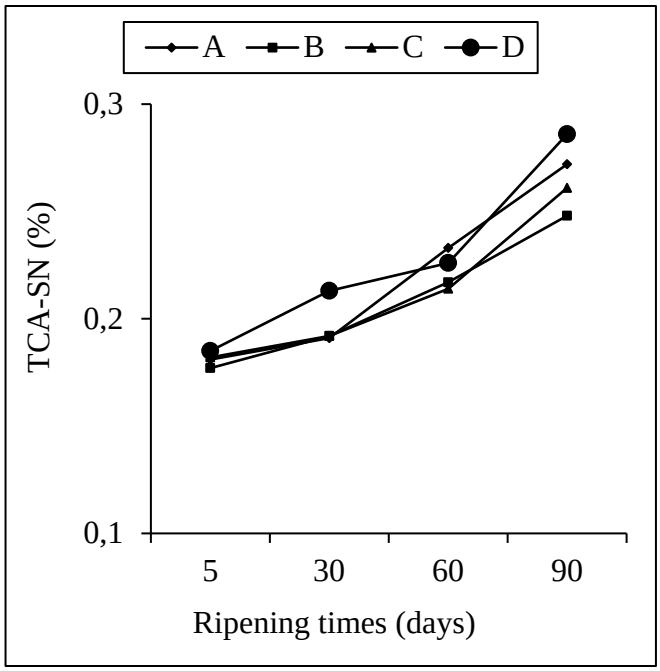

Figure 6. The changes in PTA-SN values of Kashar cheeses during ripening A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

The PTA soluble nitrogens are consist of peptides with very low weight and amino acids (Fox, 1989). The PTA-SN ratios of Kashar cheese samples were ranged in between 0.04% and 0.13%. As the highest PTA-SN ratio was found at D sample (0.08%), the lowest ratio was found at a sample. As the ripening times of cheeses increased, the PTA-SN ratio increased too. Çürük (2006) found that the TCA and TCA-SN ratio increased during ripening periods as similar results with ours.

The sensory analyses results of Kashar cheese samples were given Table 4.

Table 3. Some chemical analysis results of Kashar cheese samples

Samples	Ripening Times (days)	Acidity (%)	pH	Total Nitrogen (%)	Water Soluble Nitrogen (%)	Ripening Index (%)	TCA-TN (%)	PTA-TN (%)
A	5	0.785±0.007	5.40±0.000	3.23±0.099	0.162±0.000	5.01±0.141	0.181±0.000	0.040±0.002
	30	1.035±0.035	5.26±0.000	3.26±0.028	0.256±0.007	7.85±0.163	0.191±0.000	0.041±0.001
	60	0.940±0.057	5.23±0.007	3.36±0.007	0.324±0.020	9.67±0.615	0.233±0.004	0.048±0.001
	90	1.025±0.021	5.26±0.000	3.66±0.085	0.366±0.026	9.99±0.481	0.272±0.005	0.104±0.001
B	5	0.720±0.000	5.42±0.007	3.55±0.021	0.146±0.001	4.13±0.071	0.177±0.001	0.044±0.001
	30	0.885±0.050	5.22±0.000	3.08±0.078	0.266±0.000	8.64±0.184	0.192±0.000	0.054±0.000
	60	0.970±0.000	5.19±0.007	3.44±0.078	0.311±0.010	9.06±0.502	0.217±0.004	0.069±0.001
	90	0.795±0.050	5.18±0.007	3.23±0.064	0.358±0.001	11.10±0.184	0.248±0.001	0.104±0.001
C	5	0.760±0.000	5.44±0.007	3.56±0.064	0.158±0.002	4.44±0.007	0.182±0.002	0.039±0.002
	30	0.780±0.014	5.29±0.000	3.54±0.007	0.275±0.022	7.76±0.587	0.192±0.001	0.056±0.000
	60	0.910±0.014	5.19±0.007	3.58±0.028	0.329±0.016	9.19±0.502	0.214±0.000	0.089±0.001
	90	1.035±0.035	5.25±0.007	3.66±0.071	0.417±0.017	11.40±0.240	0.261±0.003	0.112±0.003
D	5	0.745±0.007	5.38±0.021	3.19±0.028	0.141±0.000	4.41±0.042	0.185±0.006	0.041±0.005
	30	0.765±0.007	5.28±0.000	3.56±0.014	0.264±0.002	7.40±0.085	0.213±0.010	0.064±0.000
	60	0.725±0.064	5.24±0.000	3.75±0.014	0.411±0.025	10.98±0.728	0.226±0.005	0.089±0.001
	90	0.880±0.028	5.27±0.000	3.66±0.000	0.526±0.011	14.37±0.304	0.286±0.005	0.129±0.002

*Values are means of 2 replicates ±SD

Abbreviations are: A, scalded; B, scalded and potassium sorbated; C, dry salted; D, dry salted and potassium sorbated.

Table 4. The sensory analyses results of Kashar cheese samples

Samples	Ripening Times (days)	Colour and Appearance	Texture	Taste	Odour	General acceptability	Bitterness
A	5	0.785±0.07	5.40±0.000	3.23±0.099	0.162±0.000	5.01±0.141	0.181±0.000
	30	1.035±0.035	5.26±0.000	3.26±0.028	0.256±0.007	7.85±0.163	0.191±0.000
	60	0.940±0.057	5.23±0.007	3.36±0.007	0.324±0.020	9.67±0.615	0.233±0.004
	90	1.025±0.021	5.26±0.000	3.66±0.085	0.366±0.026	9.99±0.481	0.272±0.005
B	5	0.720±0.000	5.42±0.007	3.55±0.021	0.146±0.001	4.13±0.071	0.177±0.001
	30	0.885±0.050	5.22±0.000	3.08±0.078	0.266±0.000	8.64±0.184	0.192±0.000
	60	0.970±0.000	5.19±0.007	3.44±0.078	0.311±0.010	9.06±0.502	0.217±0.004
	90	0.795±0.050	5.18±0.007	3.23±0.064	0.358±0.001	1.10±0.184	0.248±0.001
C	5	0.760±0.000	5.44±0.007	3.56±0.064	0.158±0.002	4.44±0.007	0.182±0.002
	30	0.780±0.014	5.29±0.000	3.54±0.007	0.275±0.022	7.76±0.587	0.192±0.001
	60	0.910±0.014	5.19±0.007	3.58±0.028	0.329±0.016	9.19±0.502	0.214±0.000
	90	1.035±0.035	5.25±0.007	3.66±0.071	0.417±0.017	11.40±0.240	0.261±0.003
D	5	0.745±0.007	5.38±0.021	3.19±0.028	0.141±0.000	4.41±0.042	0.185±0.006
	30	0.765±0.007	5.28±0.000	3.56±0.014	0.264±0.002	7.40±0.085	0.213±0.010
	60	0.725±0.064	5.24±0.000	3.75±0.014	0.411±0.025	10.98±0.728	0.226±0.005
	90	0.880±0.028	5.27±0.000	3.66±0.000	0.526±0.011	14.37±0.304	0.286±0.005

As seen at Table 4, color scores of sorbated samples increased during ripening, but color scores of control samples decreased during ripening. Say (2008) found that color scores changed during ripening periods. The body scores of dry salted samples were lower than that of salted in scalding water. Panelists determined that Kashar cheese samples salted as dry were harder than that of salted in scalding water. Panelists saw that dry salted samples contained the holes in and out of cheese samples during panel. Çürük (2006) found that Kashar cheese samples added themelting salt were softer during ripening periods and and texture scores decreased too. The taste scores of Kashar cheese samples varied at between 6.42 and 7.57. The taste scores given by panelists to Kashar cheese samples decreased during 90 days of ripening. The taste scores of control samples salted in scalding water were lover than that of samples salted in scalding water and sorbated. Piacquadio et al. (2001) found that the taste scores of Mozzarella cheese samples decreased during 90 days of ripening too.The founds were paralelled with research founds of our. The odour scores of sorbated Kashar cheese samples were higher than that of no sorbated samples. However, the odour scores of dry salted samples were lower than that of scalding water. Çürük (2006) found that the odour scores of Kashar cheese samples decreased as ripening periods increased as parallel our results. The bitterness score of samples decreased during ripening periods. General acceptability of of Kashar cheese samples were between 6.56 and 7.62 scores (Table 4). Generally, panelists gave higher score to general acceptibility of samples salted in scalding water. In this research, it was found the potassium sorbate added to Kashar cheese had not any unfavorable effect on the sensory properties of cheese samples. But, Aworh and Egounlety (1985) found that the potassium sorbate added to West African soft cheese was effected as unfavorable the cheese flavor. Panelists preferred the raw and cheese samples ripened for 1 months more than the other ripened periods (60 and 90 days). This state can be sourced from the unfavorable aromatic matters as free fatty acids and casein break down products formed during ripening periods for 60 and 90 days.

Conclusion

The highest mould count of Kashar cheese samples was found at control samples salted in scalding water, salted as dry and sorbated samples contained the lowest counts. For this reason, Kashar cheese must be sorbated for unmoulding. In the all cheese samples, pH value decreased the 30 and 60 days of ripening, but increased the 90 days. The highest water soluble nitrogen and ripening degree was found in Kashar cheese samples salted as dry. However, the sorbate adding to Kashar was increased the ripening level of Kashar cheese. The TCA-SN and PTA-SN ratio increased in cheese samples wich dry salted according to salted in scalding water. It was determined that there was no adverse effect of sorbate on ripening of Kashar cheese samples. Generally, panelists preferred the Kashar cheese samples ripenned for 5^{th} days and 30^{th} days of ripening time.

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References

Akbulut, N., Gonc, S., Kınık, O., Uysal, H., Akalın, S., Kavas, G. (1996). Bazı Tuzlama Yöntemlerinin Beyaz Peynir Üretiminde Uygulanabilirliği ve Peynir Kalitesine Etkileri Üzerinde Bir Araştırma 1- Duyusal ve Mikrobiyolojik Özelliklere Etkileri. *Erzincan Üniversitesi Fen Bilimleri Dergisi*, 33(1), 9-15.

Altuğ, T. (1993). Duyusal test Teknikleri. E.U. Mühendislik Fakültesi Ders Kitapları, İzmir Yayın No: 28.

Anonymous (2013). Turkish Food Codex Regulations on Food Additives. Ankara, Turkey.

Arıtaşı, C. (1990). Çeşitli Kuru Meyvelerin İlavesi ile Üretilen Vakumla Ambalajlanmış Kaşar Peynirlerinin Özellikleri Üzerine Bir Araştırma. Ph.D. Thesis, Trakya University Edirne, Turkey.

Aworh, O.C., Egounlety, M. (1985). Preservation of West African soft cheese by chemical treatment. J*ournal of Dairy Research*, 52, 187-193.

Bodyfelt, F.W., Tobias, J., Trout, G. M. (1988). The Sensory Evaluation of Dairy Products. Van Nostrand Reinhold, New York. ISBN: 9780442226855

Çürük, M. (2006). Influence of Themelting Salts Used on the Some Characteristics and Ripening Properties of Kashar Like Cheese. PhD. Thesis, Department of Food Engineering Institute of Naturel and Applied Sciences University of Çukurova, Konya, Turkey.

Diliello, L.R. (1982). Methods in Food and Dairy Microbiology. AVI Publishing Co. Inc. Westport Connt. USA. ISBN: 0870554115

Famá, L., Flores, S.K., Gerschenson, L., Goyanes, S. (2006). Physical characterization of cassava starch biofilms with special reference to dynamic mechanical properties at low temperatures. *Carbohydrate Polymers*, 66(1), 8-15.

Fox, P.F. (1989). Proteolysis during cheese manufacture and ripening. *Journal of Dairy Science*, 72(6), 1379-1400.

Gül, O., Dervişoğlu, M. (2013). Investigation of sodium benzoate and potassium sorbate content and evaluation of microbiological parameters of fresh Kashar cheeses. *Korean Journal for Food Science of Animal Resources*, 33(4), 549-554.

Güler, Z. (2005). Quantification of free fatty acids and flavor characteristics of Kasar cheeses. J*ournal of Food Lipids*, 12, 209-221.

Güven, M., Karaca O.B. (2001). Proteolysis levels of White cheeses salted and ripened in brines prepared from various salts. *International Journal of Dairy Technology*, 54(1), 29-33.

Güven, M., Karaca, O.B., Kaçar, A., Hayaloğlu, A.A., Çürük, M. (2003). Kaşar peynirlerinin proteoliz düzeyleri üzerine farklı ambalaj materyali ve olgunlaşma süresinin etkileri. GAP III. Tarım Kongresi, Şanlıurfa.

Gilliand, S.E., Sandine, W.E., Vedamuthu, E.R. (1984). Acid Producing Microorganism, Compendium of Methods for the Microbiological Examination. 2nd edn, ed. Speck, M.L. Washington D.C.: 184-196, USA. ISBN: 9780875531175

Harrigan, W.F. (1998). Laboratory Methods in Food Microbiology. Academic Press, San Diego. ISBN: 9780123260437

Jarret, W.D., Aston, J.W., Dulley, J.R. (1982). A simple method for estimating free amino acids in Cheddar cheese. *Australian Journal of Dairy Technology*, 37, 55-58.

Johnson, M., Law, B. A. (2010). The Origins, Development and Basic Operations of Cheesemaking Technology, Part 2. Technology Of Cheesemaking, 2nd Edition, Ed. Law, B. A., Tamime, M.. Wiley-Blackwell, Singapore, 68-97. ISBN : 9781405182980

Koburger, J.A., Marth, E.H. (1984). Yeasts and molds. In Compendium of Methods for the Microbiological Examination of Foods 2nd edn, ed. Speck, M.L. Washington D.C.: American Public Health Association. ISBN: 9780875531175

Koçak, C., Ersen, N., Aydınoğlu, G., Uslu, K. (1998). Ankara piyasasında satılan Kaşar peynirlerinin proteoliz düzeyi üzerinde bir araştırma. *Gıda*, 23, 247-251.

Koçak, C., Bitlis, A., Gürsel, A., Avşar, Y.K. (1996). Effect of added fungal lipase on the ripening of Kashar cheese. *Milchwissenschaft*, 51(1), 13-17.

Kristo, E., Koutsoumanis, K.P., Biliaderis, C.G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. *Food Hydrocolloids*, 22(3), 373-386.

Kurt, A., Çakmakçı, S., Çağlar, A. (2007). A Guide Book of Analysis Methods of Milk and Milk Products. Turkey: Ataturk University, Agriculture Faculty, Erzurum, Turkey.

Lau, K.Y., Barbano, D.M., Rasmusen, R.R. (1991). Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging. *Journal Dairy Science*, 74(3), 727-740.

McSweeney, P.L.H., Fox, P.F. (1997). Chemical methods for the characterization of proteolysis in cheese during ripening. *Lait*, 77, 41-76.

Messer, J.W., Behney, H.M., Leudecke, L.O. (1985). Microbiological count methods. G.H. Richardson (Ed.), Standard Methods for the Examination of Dairy Products (15th Edn), A.P.H.A, Washington, D.C., USA. ISBN: 9780875531182

Özdemir, C., Demirci, M. (2006). Selected microbiological properties of Kashar cheese samples preserved with potassium sorbate. *International Journal of Food Properties*, 9, 515-521.

Payne, M.R., Morison, K.R. (1999). A multi-component approach to salt and water diffusion in cheese. *Interantional Dairy Journal*, 9, 887-894.

Piacquadio, P., De Stefano G., Sciancalepore, V. (2001). A no brine method for Mozzarella cheese making. *Milchwissenschaft,* 56(4), 204-206.

Polychroniadou, A., Michaelidou, A., Paschaloudis, N. (1999). Effect of time, temperature and extraction method on the trichloroacetic acid-soluble nitrogen of cheese. *International Dairy Journal*, 9, 559-568.

Say, D. (2008). Effects of salt concentratıon of scalding solution and storage period on properties of Kaşar cheese, Ph.D. Thesis, Çukurova University, Adana, Turkey.

Sert, D., Ayar, A., Akın, N. (2007). The effects of starter culture on chemical composition, microbiological and sensory characteristics of Turkish Kasar cheese during ripening. *International Journal of Dairy Technology*, 4(60), 245-252.

Tarakçı, Z., Küçüköner, E. (2006). Changes on physico-chemical, lipolysis and proteolysis of vacuum-packed Turkish Kashar cheese during ripening. *Journal Central Europen Agriculture*, 7(3), 459-464.

Tatini, R.S., Hoover, D.G., Lachica, R.V.F. (1984). Methods for the isolation and enumera-tion of Staphylococcus aureusin: M.L. Speck (Ed.), Compendium of Methods for the Microbiological Examination of Foods American Public Health Association, Inc, Washington. ISBN: 9780875531175

Tfouni, S.A.V., Toledo, M.C.F. (2002). Determination of benzoic and sorbic acids in Brazilian food. *Food Control*, 13, 117-123.

TSE (2006). Kashar Cheese Standard (TS 3272). Ankara, Turkey: Turkish Standard Institute.

Türe, H., Gällstedt, M., Hedenqvist, M. S. (2012). Antimicrobial compression-moulded wheat gluten films containing potassium sorbate. *Food Research International*, 45(1), 109-115.

Uzun, Y.S. (2006). A study on viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 againts scalding and dry salting during Kaşar cheese making. M.Sc. Thesis,, Harran University, Şanlıurfa, Turkey.

Üçüncü, M. (1980). Peynircilikte sorbik asit ve kullanım olanakları. *Gıda,* 5(4), 79-87.

Üçüncü, M (2004). From A to Z Cheese Technology, Ege University Press, Izmir, Turkey, pp. 948-969. ISBN: 975-98951-2-9

Üçüncü, M. (2010). Süt ve Mamulleri Teknolojisi. Ege Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü, 571s, İzmir. ISBN: 978-975-98951-3-6

Yalman, M., Güneşer, O., Yüceer, Y.K. (2017). Evaluation of Some Physical, Chemical and Sensory Properties of Kasar Cheese and Its Processed and Analogue Types. *Tarım Bilimleri Dergisi,* 23(1), 63-75.

Yangılar, F., Oğuzhan Yıldız, P. (2016). Casein/natamycin edible films efficiency for controlling mould growth and on microbiological, chemical and sensory properties during the ripening of Kashar cheese. *Journal of the Science of Food and Agriculture*, 96(7), 2328-2336.

Yılmaz, F., Dağdemir, E. (2012). The effects of beeswax coating on quality of Kashar cheese during ripening. *International Journal of Food Science and Technology*, 47, 2582-2589.

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ENHANCEMENT OF BIOAVAILABLE MICRONUTRIENTS AND REDUCTION OF ANTINUTRIENTS IN FOODS WITH SOME PROCESSES

Müge Hendek Ertop[1], Müberra Bektaş[2]

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[1] Department of Food Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kuzeykent, 37000, Kastamonu, Turkey

[2] Department of Food Engineering, Faculty of Engineering and Natural Science, Gümüşhane University, Bağlarbaşı, 29000 Gümüşhane, Turkey

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Correspondence:

Müge HENDEK ERTOP

E-mail: muge_ertop@hotmail.com

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ABSTRACT

The most of plant foods, nuts and cereals contain antinutrient compounds. They reduce to mineral bioavailability and protein absorption of foods thanks to their chelating properties. They causes to micronutrient malnutrition and mineral deficiencies. The micronutrient malnutrition is a widespread global health problem not only in developing but also in many countries. Increasing micronutrient intake in food through food processing based approaches is a sustainable method of prevention of micronutrient malnutrition which should be achieved through food diversification. There are traditional and technological methods that provide reducing of antinutrient compounds.The pretreatment and processing techniques as soaking, fermentation, germination, debranning, and autoclaving are even traditional methods which use generally in consumption of foods.Removing antinutrients, the bioavailability of some cation (Ca, Fe and Zn) and the absorption of proteins make to increase and consequently nutrition value of food increase. It is possible to reduce antinutrient factors by using domestic or industrial basic food processing techniques alone or in combination.This review focused on various methods to reduce antinutrients in food such as phytic acid, tannin, and oxalate in food grain to improve nutritional quality of foods.

Keywords: Micronutrients, Antinutrients, Digestibility, Bioavailability

Introduction

The malnutrition influences more than half of the world population, especially in developing countries in which the plants to be a major source of food. The deficiencies of micronutrients such as minerals and vitamins have caused to be most serious health problems (Jorge *et al.*, 2008). The solely total micronutrient content is not important in foods, their bioavailability is more effective factor than their level. In unrefined foods the low bioavailability of minerals causes metabolic disorder related to these nutritional factors. Therefore improving the nutritional value of such type of foods will improve the nutritional status of the population (Steiner *et al.*, 2007; Gupta *et al.*, 2015).

Phytate has long been recognized as an antinutritional factor affecting the bioavailability of major minerals such as Ca and trace ones such as Fe, Cu, Zn and Mn. Other antinutrients of importance in foods are tannins, polyphenols, oxalats and tripsins. The known that they limit the bioavailability of food materials (Eltayeb *et al.*, 2007). Decreasing of antinutritional factors is very advantageous, due to their influences on nutrition. However, many antinutrients besides their primary effects on the bioavailability of nutrients may also be toxic beyond a certain dose, for example oxalate or cyanogenic acid. Therefore interest has grown to reduce their antinutritional effects nowadays (Novak and Haslberger, 2000).

Soaking, dehulling, cooking and fermentation are important traditional methods used to reduce antinutrients. Furthermore germination and fermentation enhance the nutritional value of cereals ad legumes by causing significant changes in chemical composition and elimination of antinutritional factors (Abdelrahaman *et al.*, 2005).

The aim of this review was to evaluate the effect of processing methods which can be decrease the level of antinutrients such as phytic acid, polyphenols and tannin which are mostly found in food materials.

Antinutrients in Foods

The compounds such as phytic acid, tanin and polyphenols found in leguminous, vegetables and cereal grains are known as anti-nutritional factors affecting the bioavailability of proteins, minor minerals such as Zn, Fe, Cu and major minerals such as Ca and P. Antinutrients reduce the maximum utilization of nutrients (especially proteins, vitamins or minerals), and as a consequence they obstruct an optimal bioavailibility of the nutrients present in a food and decrease its nutritive value (Eltayeb *et al.*, 2007).

Phytic acid myoinositol is 1,2,3,4,5,6-hexa dihydrogen phosphate. It is the major storage form of phosphorous comprising 1–5% by weight in cereals, nuts and legumes (Vats and Banerjee, 2004). Moreover it include 50–85 % of total phosphorous in plants (Reddy *et al.*, 1982). Cereals, legumes, oil seeds, hard shelled fruits, which are necessary in human nutrition, are the considerable phytic acid sources. They represents approximately 40 % and 60 % of total calorie intake for daily human diet (Schlemmer *et al.*, 2009). Especially cereals and cereal products are rich in phytic acid content. In cereal grains such as wheat and rice, it is generally found in bran fraction such as aleurone layer and pericarp, in corn it is seen in endosperm (Gupta *et al.*, 2015). It was reported that the phytic acid concentration in wheat germ and wheat bran are 1.1–3.9 % and 2.0–5.3 % respectively (Kasim and Edwards, 1998). The phytic acid content is upto 8.7 % in rice bran (Lehrfeld, 1994; Zhang and Bai, 2014). Therefore, the rafination of the cereals significantly effect to phytic acid content (Suma and Urooj, 2014). The phytic acid content varies from approximately 1.0–5.4 % of the other group oilseeds which includes soybeans, sesame seeds, sunflower kernels, linseeds and rape seeds (Lolas *et al.*, 1976). Another group of foods contained phytic acid is nuts such as walnuts, almond, in which phytic acid content ranged from approx. 0.1–9.4 % (Chen, 2004; VenktachalamandSathe, 2006; Schlemmer *et al.*, 2009). Phytic acid acts blocking the absorption of minerals such as Fe, Zn, and Ca. For that reason it also named antinutritive agent. This binding phenomonia caused to insoluble salt form with poor bioavailability of minerals (Urbano *et al.*, 2000; Feil, 2001).

Another antinutrient groups in foods are tannins and free phenolics. In terms of human nutrition, the content of both tannins and total free phenolics are not desirable for human consumption. Whereas phytic acid reduces the bioavailability of some essential minerals, tannins inhibit the digestibility of protein (Rehman and Shah, 2001). It was reported that, phenolic compounds decreased the digestibility of proteins, carbohydrates and the bioavailability of vitamins such as vitamin B12 and minerals (Liener, 1994). They also decreases the activity of digestive enzymes such as trypsin, chymotrypsin lipase and α – amylase.

Oxalic acid is a dicarboxylic acid generally found in plants and animals. Besides dietary intake it in the human body may also be derived from metabolism of ascorbic acid and glyoxylate. The previously conducted studies reported that star fruit, spinach, amaranth, bamboo shoot, ginger, almond, cashew, pine nut, hazel and peanut contained high levels of

oxalateas. Moreover, water spinach, Chinese wolfberry, black glutinous rice, dragon fruit, rice bean, abalone fruit and Chinese torreya fruit were also presented as high oxalate-foods (Ruan *et al.*, 2013). The consumption of high amount of oxalate could be fatal, because of oxalosis or the formation of calcium oxalate deposits in vital tissues or organs of the body (Sanz and Reig, 1992). The oxalate intake should be to less than 40–50 mg per day recommended by the American Dietetic Association, (2005) for the patients with kidney stone problems.

Technological Processes and Changes in Antinutrients Content

Most of the anti nutritive substances become ineffective or their level can be reduced with simple treatments such as heating, soaking, germination or autoclaving.

Milling and Debranning

Milling is the most commonly used method to remove the bran layer from grains. Furthermore this technique removes the antinutrients such as phytic acid but also has major disadvantages as it also removes major parts of minerals and dietary fibers (Gupta *et al.*, 2015).

Soaking

The soaking is an easy method used generally in daily life and an important method helped in germination and fermentation of cereals. During soaking, phytase enzyme activates and effects to antinutrient compounds. Soaking process has both physical and chemical positive effects in structure of foods. For this reason, cereals such as chickpea, wheat and barley used to product in daily life should be consumed after soaked for a while (Gupta *et al*., 2015).

This method is the complete submergence of grains in water for a certain soaking period which results in the activation of endogenous phytases. The endogenous phytases are present in grains naturally. So by activation of these enzymes with several treatment such as soaking it has been reported that significant amount of phytic acid content in grains have been removed. Soaking is widely applied and most important method in germination and fermentation process of cereals. Furthermore it is a pretreatment before cooking for all grains. Soaking of cereals with endogenous or exogenous phytase increases in vitro solubility of minerals such as Fe and Zn by 2–23% (Lestienne *et al.*, 2005).

In this method, the soaking water's heat and soaking time act significant. In a study conducted by Greiner and Konietzny (2006), soaking at temperature between 45°C and 65°C and pH value between 5 and 6 a considerable percentage of phytate was hydrolysed. The grains and beans soaking is quite effective for increasing in mineral and protein bioavailability as well as reduction of phytic acid (Coulibaly *et al.*, 2011). The soaking duration and the combination with other treatments such as cooking are more effective that the only usage of soaking. As soaking time increased from 2h to 12 h phytic acid content in chick pea decrease by 47.4 % to 55.71 % has been reported (Ertas and Turker, 2014).

On the other hand disadvantages of treatment, it can be disappearing of water soluble protein and minerals.

Fermentation

Fermentation is the one of the important processes that decrease the levels of antinutrients in food grains and increase minerals extractability, *in-vitro* protein digestibility and nutritive value of grains. The reduction in phytic acid during fermentation could be attributed to the action of the enzyme phytase released by microorganisms' fermentation. Therefore cereals based foods certainly should be consumed after fermentation treatment. It reduces amounts of phytic acid, tannin, polyphenols with fermentation treatment and it increase food's mineral bioavailability and digestibility (Gupta *et al*., 2015).

Fermentation is the one of the processes that decrease the levels of antinutrients in food grains and increase minerals extractability (Badau *et al*., 2005). Fermentation of food grains improves bioavailability of minerals and proteins. The phytic acid is present in grains in the form of complexes with metal cations such as Zn, Fe, Ca and proteins. The enzymatic degradation of phytic acid requires an optimum pH (generally below pH 4.5) which can be provided by natural fermentation such as be in sourdough fermentation (Hayta and Hendek Ertop, 2017). The degradation of phytic acid can increase the amount of soluble of minerals. It have been reported that fermentation of millet grain for 12 h and 24 h could reduce the food inhibitors, phytic acid and tannins (Coulibaly *et al*., 2011). The natural fermentation also named as spontan fermentation can achieve a large reduction in phytic acid in cereals and legumes by the action of microbial as well as grain phytases. Because, the phytases which is based on grain and microbiota act on phytic acid during fermentation. The combination of fermentation with other treatments such as germination are more effective that the only usage of fermentation. There was recorded that 88.3% reduction in phytate content when germinated pearl millet were fermented with mixed pure cultures of *Saccharomyces diasticus*, *S. cerevisiae*, *Lactobacillus brevis* and *L.*

fermentum at 30 °C for 72 h (Kaur *et al.*, 2014). Abdelrahaman *et al*. (2005) reported that, germination and fermentation enhance the nutritional value of pearl millet by causing significant changes in chemical composition and elimination of antinutritional factors.

Germination

Germination is highly effective method to reduction of phytic acid content by up to 40% (Masud *et al*., 2007). During the germination process, the endogenous enzyme activity which has phytate degrading ability increases. This condition provide to degradation and decline antinutrients as phytic acid. In non-germinated cereal and legume grains have a little endogenous activity (Greiner and Konietzny, 2006). In a study conducted by Marshall *et al.* (2011), cereal grains were screened for phytic acid content and found that germination for 10 days resulted in a significant reduction (p<0.05) in the phytate contents of all cereal grains screened.

Autoclaving and Cooking

Autoclaving is heat treatment application. Together with application heat treatment to cereals or other vegetable goods, acidity increase and phytase enzyme activate. The most of foods become usefull and healty with heat treatment application in daily diet. All legumes and some cereals are usually cooked either by simple boiling or in a pressure cooker for their consuming. The literature is reported that simple boiling improves the nutritional quality of food grains due to reduction in antinutrients (Rehman and Shah, 2005).

The phytic acid content is greatly reduced during cooking and soaking (Vellingiri and Hans, 2010). Together soaking and cooking are much more effective to reduce phytic acid level than only soaking for a short duration (Vidal-Valverde *et al.*, 1994). In a study, autoclave and microwave treatments decreased phytic acid content as they also increased total mineral content and HCl-extractability of minerals in whole wheat bread (Mustafa and Adem, 2014).

It has been observed, by previous studies, that different cooking methods improve the nutritional quality of food legumes to various extents (Nielson, 1991; Chi-Fai *et al*., 1997). Singh (1993) reported that improvement in protein quality of pigeon was obtained after the partial removal of polyphenols as a result of a simple boiling method. In another study, it was founded that pressure cooking was more effective than ordinary cooking in reducing the antinutrients of black grams and mung beans (Kataria *et al.*, 1989). Moreover it was revealed that the boiling and autoclaving in water improved the protein quality of winged beans due to reduction in the levels of antinutrients (Kadam *et al*., 1987). Rehman and Shah (2001), observed an improvement in protein digestibility of black grams due to removal of tannins after pressure cooking.

Furthermore, it was reported by earlier studies that cooking treatment improves the digestibility of starch through gelatinization and destruction of antinutrients (Mbofung *et al.*, 1999; Rehman *et al.*, 2001). The improvement in starch digestibility can based on due to hydrolysis of starch as a result of heat treatments. According to another phenomonia, partial removal of tannins and phytic acid probably is creates a large space within the matrix, which increased the susceptibility to enzymatic attack and consequently improves the digestibility of protein and starch after the cooking process (Rehman and Shah, 2005).

It has been shown that a significant difference in oxalate content was obtained according to cooking methods. For example, when the taro samples were boiled in water for 40 min, oxalate content decreased by at least 47%. However, when the samples were baked at 180 °C for 40 min, this treatment did not bring about a significant change in the oxalate level (Savage and Martensson, 2010).

It was reported that the toasting process resulted in a significant reduction in trypsin-inhibitor activity of the toasted meal of soybean relative to the seed (Novak and Haslberger, 2000).

According to different studies, among the various common processing methods, the autoclaving treatment was found to be more effective in reducing various antinutritional compounds (Shimelis and Raksihit, 2007; Vadivel *et al.*, 2007; Doss *et al.*, 2011). Doss *et al.* (2011), reported that the autoclaving, cooking and soaking processing methods were found to reduced significant levels of various antinutritioanl compounds such as total free phenolic respectively.

Conclusion

The processing methods of foods can significantly decrease antinutrients. Genetic improvement as well as several pretreatment methods such as fermentation, soaking, germination also improves nutritional quality. Further decrease in antinutritional factors can be obtained by the usage of various fermentation methods such as spontaneus fermentation named as sourdough method of the processed grains. Cost effective processes for commercial and industrial productions should be developed. Future researchs are needed to determine the optimal processing conditions and to appropriate delivery of phytase enzyme to foods.

There are close negative corelation between the level of antinutrients and the biovailability of micronutrients. For that reason the studies for determination of *in vitro* bioavailability of micronutrients in foods should be also done.

Nowadays there has been consumer's tendency towards food types that are produced with unrefined grains, cereals and legumes such as whole wheat flour or wheat bran. In this respect, the bioavailability of foods containing different types of cereals and grain fractions has been expected to improve by the use of this processes.

References

Abdelrahaman, S.M., El Maki, H.B., Babiker, E.E., El Tinay, A.H. (2005). Effect of malt pretreatment followed by fermentation on antinutritional factors and HCl- Extractability of minerals of pearl millet cultivars. *Journal of Food Technology*, 3, 529-534.

American Dietetic Association (2005). Urolithiasis /urinary stones. In, ADA Nutrition Care Manual. Chicago IL. USA, 483-486.

Badau, M.H., Nkama, I., Jideani, A.I. (2005). Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. *Journal of Food Chemistry*, 92, 425-435.

Chen, Q.C. (2004). Determination of phytic acid and inositol pentakis phosphate in foods by HPLC. *Agricultural Food Chemistry*, 52, 4604-4613

Chi-Fai, C., Peter, C.-Kc., Shing, W.Y. (1997). Effect of cooking on content of amino-acids and antinutrients in the Chinese indigenous legume seed. *Journal of the Science of Food and Agriculture*, 75, 447-452.

Coulibaly, A., Kouakou, B. Chen, J. (2011). Phytic acid in cereal grains: Healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, 1, 1-22.

Doss, A., Pugalenthi, M., Vadivel, V. G., Subhashini, G., Anitha Subash, R. (2011). Effects of processing technique on the nutritional composition and antinutrients content of under –utilized food legume *Canavalia ensiformis* L.DC. *International Food Research Journal*, 18(3), 965-970

Greiner, R., Konietzny, U. (2006). Phytase for food application. *Food Technology Biotechnology*, 44, 125–140.

Hayta, M., Hendek Ertop, M. (2017). Optimization of sourdough bread incorporation into wheat bread by response surface methodology: Bioactive and nutritional properties, *International Journal of Food Science and Technology*, 52(8), 18258-1835.

Eltayeb, M.M., Hassn, A.B., Sulieman, M.A., Babiker, E.E. (2007). Effect of processing followed by fermentation on antinutritional factors content of pearl millet (*Pennisetum glaucum* L.) cultivars. *Pakistan Journal of Nutrition*, 6 (5), 463-467

Ertas, N., Turker, S. (2014). Bulgur processes increase nutrition value: possible role in in-vitro protein digestability, phytic acid, trypsin inhibitor activity and mineral bioavailability. *Journal of Food Science Technology*, 51(7), 1401-1405.

Feil, B. (2001). Phytic acid. *Journal of New Seeds*, 3, 1–35.

Gupta, R.K., Gangoliya, S.S., Singh, N.K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, 52 (2), 676–684.

Jorge E.M., Wolfgang, H.P., Peter, B. (2008). Biofortified crops to alleviate micronutrient malnutrition. *Current Opinion Plant Biology*, 11, 166-170.

Kadam, S.S., Smithard, R.R, Eyre, M.D., Armstrong, D.G. (1987). Effect of heat treatment on ntinutritional factors and quality of protein in winged beans. *Journal of Science of Food and Agriculture*, 39, 267-275.

Kasim, A.B., Edwards, H.M.J. (1998). The analysis of inositol phosphate forms in feed ingredients. *Journal of the Science of Food and Agriculture*, 76, 1-9.

Kataria, A., Chauhan, B.M., Punia, D. (1989). Antinutrients in amphidiloids (blackgram & mung bean) varietal differences and effect of domestic processing and cooking. *Plant Food and Human Nutrition*, 39, 257-266.

Kaur, K.D., Jha, A., Sabikhi, L., Singh, A.K. (2014). Significance of coarse cereals in health and nutrition: a review. *Journal of Food Science and Technology*, 51(8), 1429-1441.

Lehrfeld, J. (1994). HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problems and solutions. *Journal of Agricultural Food Chemistry*, 42, 2726–2731.

Lestienne, I., Caporiccio, B., Besancon, P., Rochette, I., Treche, S. (2005). Relative contribution of phytates, fibers and tannins to low iron and zinc in vitro solubility in pearl millet (*Pennisetum glaucum*) flour and grain fractions. *Journal of Agricultural Food Chemistry*, 53, 8342-8348.

Liener, I.E. (1994). Implications of antinutritional components in soybean foods. *CRC Critical Reviews in Food Science and Nutrition*, 34, 31-67.

Lolas, G.M., Palamidids, N., Markakis, P. (1976). The phytic acid—total phosphorus relationship in barley, oats, soybeans and wheat. *Cereal Chemistry*, 53, 867-871.

Marshall, A.A., Samuel, J.E., Mary, U.E., Inegbenose, G.I. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice, maize, millet, sorghum and wheat. *Journal of Food Science and Technology*, 48, 724-729.

Masud, T., Mahmood, T., Latif, A., Sammi, S., Hameed, T. (2007). Influence of processing and cooking methodologies for reduction of phytic acid content in wheat (*Triticum aestivum*) varieties. *Journal of Food Processing and Preservation*, 31, 583-594.

Mbofung, C.M.F., Rigby, N., Waldron, K. (1999). Use of two varieties of hard-to-cook beans and cowpeas in the processing of Koki (a steamed legume product). *Plant Food and Human Nutrition*, 54, 131-150.

Mustafa, K.D., Adem, E. (2014). Comparison of autoclave, microwave, IR and UV-stabilization of whole wheat flour branny fractions upon the nutritional properties of whole wheat bread. *Journal of Food Science and Technology*, 51(1), 59-66.

Nielson, S.S. (1991). Digestibility of legume protein. *Journal of the Food Technology*, 45, 112–118.

Novak, W.K., Haslberger, A.G. (2000). Substantial equivalence of antinutrients and inherent plant toxins in genetically modifed novel foods. *Food and Chemical Toxicology*, 38, 473-483.

Reddy, N.R, Sathe, S.K., Salunkhe, D.K. (1982). Phytases in legumes and cereals. *Advances in Food Research*, 82, 1-92.

Rehman, Z.U., Salariya, A.M., Yasin, M., Zafar, S.I. (2001). Thermal heat processing effects on in vitro protein digestibility of chickpea (*Cicer arietinum*). *Pakistan Journal of Science and Research*, 53, 75-77.

Rehman, Z.U., Shah, W.H. (2001). Tannin contents and protein digestibility of black grams (*Vigna mungo*) after soaking and cooking. *Plant Food and Human Nutrition*, 56, 265-273.

Rehman, Z.U., Shah, W.H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry*, 91: 327-331.

Ruan, Q.Y., Zheng, X.Q., Chen, B.L., Xiao, Y., Peng, X.X., M, Leung, D.W., Liu, E.E. (2013). Determination of total oxalate contents of a great variety of foods commonly available in Southern China using an oxalate oxidase prepared from wheat bran. *Journal of Food Composition and Analysis*, 32, 6-11.

Sanz, P., Reig, R. (1992). Clinical and pathological findings in fatal plant oxalosis. *The American Journal of Forensic Medicine and Pathology*, 13, 342-345.

Savage, G.P., Martensson, L. (2010). Comparison of the estimates of the oxalate content of taro leaves and corms and a selection of Indian vegetables following hot water, hot acid and in vitro extraction methods. *Journal of Food Composition and Analysis*, 23, 113-117.

Schlemmer, U., Frolich, W., Prieto, R.M., Grases, F. (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition & Food Research*, 53, 330-375

Shimelis, E.A., Rakshit, S.K. (2007). Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris L.*) varieties grown in East Africa. *Food Chemistry*, 103, 161-172.

Singh, U. (1993). Protein quality of pigeon pea as influenced by seed polyphenols and cooking process. *Plant Food and Human Nutrition*, 43, 171-179.

Steiner, T., Mosenthin, R., Zimmermann, B., Greiner, R., Roth, S. (2007). Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Animal Feed Science and Technology*, 133, 320-334.

Suma, P.F., Urooj, A. (2014). Nutrients, antinutrients and bioaccessible mineral content (invitro) of pearl millet as influenced by milling. *Journal of Food Science and Technology*, 51(4), 756-761.

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function?, *Journal of Physiology and Biochemistry*, 56, 283-294.

Vadivel, V., Pugalenthi, M., Megha, M. (2008). Biological evaluation of protein quality of raw and processed seeds of gila bean (*Entada scendens Benth*.) *Tropical and Subtropical Agroecosystem*, 8, 125-133.

Vats, P., Banerjee, U.C. (2004). Production studies and catalytic properties of phytases (myo-inositol-hexakis-phosphate phosphohydrolases): an overview. *Enzyme and Microbial Technology*, 35, 3-14.

Vellingiri, V., Hans, K.B. (2010). Effect of certain indigenous processing methods on the bioactive compounds of ten different wild type legume grains. *Journal of Food Science and Technology*, 49, 673-684.

Venktachalam, M., Sathe, S.K. (2006). Chemical composition of selected edible nut seeds. Journal of Agricultural, *Food Chemistry*, 54, 4705-4714.

Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M.J., Ruiz, R., Bacon, J. (1994). Effect of processing on some antinutritional factors of lentils. *Journal of Agricultural Food Chemistry*, 42, 2291-2295.

Zhang, H.W., Bai, X.L. (2014). Optimization of extraction conditions for phytic acid from rice bran using response surface methodology and its antioxidant effects. *Journal of Food Science and Technology*, 51(2), 371-376.

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GIDALARDA BULUNAN BİYOJEN AMİNLERİN ÖNEMİ VE DETOKSİFİKASYON MEKANİZMALARI

Sadiye Akan, Mustafa Kemal Demirağ

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Ege Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Bornova/ İzmir, Türkiye

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Correspondence:

Sadiye AKAN

E-mail: sadiyeakan@gmail.com

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ÖZ

Günümüzde gıdalarda iz miktarlarda bulunan fakat sağlık açısından önemli etkiler gösteren bileşiklerin belirlenmesi üzerine yapılan çalışmalar devam etmektedir. Biyojen aminler de özellikle proteince zengin gıdalarda ve fermente gıdalarda mikrobiyal dekarboksilasyon veya aldehitlerin ve ketonların deaminasyonu sonucu oluşan ve sağlık açısından birçok etkileri olan bileşikler olarak bu araştırmalara konu olmaktadır. Biyojen aminler vücutta birçok fizyolojik fonksiyonun düzenlenmesinde görev almakla birlikte vücutta yüksek konsantrasyonlarda bulunduklarında ise toksik etki gösterebilmektedirler. Biyojen aminler vücut sisteminde amino oksidaz enzimleri tarafından detoksifiye (katabolize) edilebilmekte olup, bazı durumlarda bu enzimlerin yeterince aktivite göstememesi sonucu bu aminler vücutta toksik etki gösterebilmektedirler. Bu derlemede, biyojen aminlerin fizyolojik etkileri, toksik etkileri ve detoksifikasyon mekanizmaları güncel literatürler incelenerek tartışılmıştır.

Anahtar Kelimeler: Biyojen aminler, Fizyolojik etki, Toksik etki, Detoksifikasyon

ABSTRACT

THE IMPORTANCE OF BIOGENIC AMINES IN THE FOODS FOR HEALTH AND THEIR DETOXIFICATION MECHANISMS

Nowadays, the researches about determination of compounds that are present at trace amounts in foods and have significant effects on health have been proceeded. Biogenic amines, which are formed by microbial decarboxylation or deamination of aldehites and ketones, especially in the foods rich in proteins and fermented foods and have many effects on human health, subject to the so-called researches. Biogenic amines play important roles in the regulation of many physiological functions in the body, however excessive intake of biogenic amines could lead to toxicological effects. These amines are detoxified (catabolized) by amine oxidase enzymes in the body, however biogenic amines may cause toxicological effects in cases enzymes at issue are inactivated by some substances. In this review, the physiological and toxicological effects of biogenic amines and their detoxification mechanisms are discussed.

Keywords: Biogenic amines, Physiological effect, Toxicological effect, Detoxification

Giriş

Biyojen aminler bitki, hayvan ve mikroorganizma metabolizması tarafından özellikle proteince zengin ve fermente tip gıdalarda amino asitlerin dekarboksilaz enzimleriyle dekarboksilasyonu veya aldehit ve ketonların amino asit transaminaz enzimi ile deaminasyonu sonucu oluşan düşük moleküler ağırlıklı organik bileşikler olarak tanımlanmaktadırlar (Brink vd., 1990; Özdestan ve Üren, 2012; Silla-Santos., 1996). Biyojen aminler kimyasal molekül yapılarına göre alifatik (putresin, kadaverin, spermin, spermidin, agmatin), aromatik (tiramin, feniletilamin) ve heterosiklik (histamin, triptamin) bileşikler olarak ya da içerdikleri azot sayısına göre monoaminler (β- feniletilamin, tiramin), diaminler (histamin, triptamin, putresin, kadaverin) ve poliaminler (agmatin, spermin, spermidin) olarak sınıflandırılabilmektedirler (Halász vd., 1994; Smith, 1980). Aminler, sentezlenme mekanizmalarına göre ise "doğal poliaminler" ve "biyojen aminler" olarak da ayrılabilmektedirler (Azim, 2002; Bardócz, 1995; Til vd., 1997).

Biyojen aminler üzerine yapılan çeşitli çalışmalarda biyojen aminlerin taze gıdalarda düşük konsantrasyonlarda veya hiç bulunmadıkları belirlenmiş olmakla birlikte; balık ve ürünleri, et ve ürünleri, süt ve ürünleri gibi yüksek proteinli gıdalar ile şarap, bira, sebzeler, meyveler gibi düşük proteinli gıdalar ve kabuklu yemiş, çikolata ve fermente gıdalarda da toksisiteye yol açabilecek yüksek konsantrasyonlarda bulunabildikleri belirtilmektedir (Askar ve Treptow, 1986; Brink vd., 1990; Flick vd., 2004).

Gıdalarda oluşan en önemli biyojen aminler histamin, β-feniletilamin, tiramin, triptamin, putresin, kadaverin, agmatin, etilamin, etanolamin, spermin ve spermidindir (Özdestan ve Üren, 2012; Stadnik ve Dolatowski, 2010). Gıdalarda oluşan biyojen aminler, gıdaların bozulmaları, tazelikleri ve gıda güvenliği ile yakından ilgili olup, histamin, putresin, kadaverin, spermidin ve tiramin gibi biyojen aminler ve bunların konsantrasyonları gıdalardaki tazeliğin gösterilmesinde indikatör maddeler olarak kullanılabilmektedirler. Bu sebeple biyojen amin analizleri temel olarak hammadde, ara ürünler ve son ürünlerin kalite kontrolleri ile fermantasyon sürecinin izlenmesi, proses kontrolü, araştırma ve geliştirme gibi aktivitelerde önemli olmaktadır (EFSA, 2011; Gürbüz, 2002; Önal, 2007; Uylaşer ve Konak, 2004).

Biyojen aminlerin insan ve hayvanların çeşitli fizyolojik fonksiyonlarının düzenlenmesinde görev aldıkları ancak vücuda yüksek miktarlarda biyojen amin alınması ile vücudun doğal amin detoksifikasyon kapasitesi aşılabildiğinden, bu aminler vücutta yeterince detoksifiye edilememekte ve toksik etkiler gösterebilmektedirler (Karovičová ve Kohajdová, 2005; Özoğul vd., 2004; Yerlikaya ve Gökoğlu, 2002). Ancak gıdalarda bulunan biyojen aminlerin gösterdiği toksik etkiler sadece ortamda bulunan tek bir biyojen amininin varlığına bağlı olmamakta, aynı zamanda ortamda bulunan diğer aminlerin sinerjistik etkisine de bağlı olmaktadır (Alvarez ve Moreno-Arribas, 2014). Çeşitli çalışmalardan elde edilen bilgiler tek bir biyojen aminin veya birden çok biyojen aminin birlikte bulunduğu durumlarla ilgili olarak sağlık açısından kantitatif risk analizini yapmak için yeterli bulunmamaktadır. EFSA'da histamin ve tiramin için bir kişinin bir öğünde tüketebileceği ve sağlık üzerinde olumsuz etkiler göstermeyen maksimum miktarlar belirtilmiştir. Buna göre; sağlıklı bireyler için 50 mg histamin en fazla tüketebilecek miktar iken, histamin intoleransı olan bireylerde bu miktar tespit edilebilen limit değerlerinin çok daha altında kalmaktadır. Tiramin için ise bu miktarlar; monoamino oksidaz inhibitörü (MAOI) ilaçlarını kullanmayan sağlıklı bireyler için 600 mg, üçüncü nesil MAOI ilaçları kullanan bireyler için 50 mg ve klasik MAOI ilaçları kullanan bireylerde ise 6 mg olarak belirlenmiştir (EFSA, 2011).

Biyojen aminler sağlık üzerinde olumsuz etkiler gösterebildiğinden, gıdalarda biyojen amin oluşumunun önlenmesi önem taşımaktadır. Modifiye atmosferde ambalajlama, yüksek basınç uygulamaları, ışınlama uygulamaları, gıdaların üretiminde amin negatif starter kültürlerin kullanılması ve gıdalara koruyucu maddelerin eklenmesi gibi uygulamalarla gıdalarda biyojen aminlerin oluşumu önlenebilmektedir (Mohamed ve Toliba, 2013; Naila vd., 2010).

Biyojen Aminlerin Sağlık Açısından Önemi

Biyojen aminlerin fizyolojik etkileri

Farklı tipteki birçok biyojen aminin vücutta önemli ve çeşitli biyolojik etkilerinin mevcut olduğu, özellikle ökaryotik hücrelerde; hormonların, alkaloidlerin, nükleik asitlerin ve proteinlerin sentezlenmesinde azot kaynağı olarak öncül maddeler olarak kullanıldığı, organizmada vücut sıcaklığının düzenlenmesi, kan basıncının azaltılması ve arttırılması gibi birçok işlevlerin yerine getirilmesinde rol oynadıkları belirlenmiştir (Çolak ve Aksu, 2002; Karovičová ve Kohajdová, 2005; Spano vd., 2010). Prokaryotik hücrelerde ise biyojen aminlerin fizyolojik rollerinin, biyojen amin oluşumuyla ortam pH'sının yükselmesi ve böylece mikroorganizmaların asidik ortam etkisinden korunması olduğu belirtilmektedir (Alper ve Temiz, 2001; Lee vd., 2007; Rhee vd., 2002). Bazı araştırmalarda ayrıca, *Escherichia coli* bakterisini oksidatif strese karşı koruyan *oxyR* geninin putresin konsantrasyonuyla artmakta olduğu belirtilmiştir (Lee vd., 2007; Rhee vd., 2002; Spano vd., 2010).

Etki şekillerine göre biyojen aminler, vazoaktif (damar sistemine etkili) ve fizyoaktif (sinir sistemine etkili) aminler olarak ayrılabilmektedirler (McCabe-Sellers vd., 2006). Tiramin, triptamin ve feniletilamin gibi vazoaktif aminler, damar sistemlerini direkt veya indirekt olarak etkilerken; histamin, putresin ve kadaverin gibi fizyoaktif aminler de merkezi sinir sistemindeki nörotransmitterleri etkilemektedirler (Cardozo vd., 2011). Katekolaminler, indolaminler ve histamin gibi aminlerin bazıları insanlarda özellikle sinir sisteminde ve kan basıncının kontrol edilmesi gibi metabolik fonksiyonlarda önemli etkiler gösterebilirken, feniletilamin ve tiramin kan basıncını arttırmakta, histamin ise aksine kan basıncını düşürücü etki gösterebilmektedir (Halász vd., 1994; Stratton vd., 1991; Taylor, 1986). Putresin, kadaverin ve spermidin gibi bazı biyojen aminler serbest radikal süpürücü olarak davranabilirken tiramin ise amino ve hidroksil gruplarının etkisiyle konsantrasyonunun artışıyla bağlantılı olarak önemli ölçüde antioksidan aktivite gösterebilmektedir. Spermin de amino grubundan hidrojen vericisi aracılığıyla tokoferoksil radikalinden tokoferol sentezleyebilmekte ve böylece antioksidan aktivite gösterebilmektedir (Karovičová ve Kohajdová, 2005). Putresin, spermidin ve spermine genellikle hayvanlar ve bitkilerde rastlanırken; birçok bakteride ise putresin ve spermidine rastlanılmaktadır (Halász vd., 1994; Yerlikaya ve Gökoğlu, 2002).

Tüm yaşayan hücrelerde biyojen aminler fizyolojik ve metabolik prosesler sırasında sentezlenmekte ve insan vücudunda; yenilenme, normal metabolik fonksiyonların aktivitesinin sürdürülmesinde, bağırsaklardaki immünolojik sistemde ve vücuttaki her organın metabolizması için de gerekli maddeler olarak karşımıza çıkmaktadırlar (Çolak ve Aksu, 2002; Halász vd., 1994; Karovičová ve Kohajdová, 2005; Tassoni vd., 2004). Poliaminlerden, özellikle spermidin temel olarak sindirim sisteminde gıda alerjenlerinin alımını düzenleyerek çocuklarda alerjinin önlenmesinde görev alırken; serotonin ise merkezi sinir sisteminde ve bağırsaklarda sentezlenip, merkezi sinir sisteminde nörotransmitter olarak duyguların düzenlenmesi (uyku, susama, açlık, ruh hali ve cinsel aktivite) gibi çok sayıda fizyolojik fonksiyonun yapılandırılmasında görev almaktadır (Kalač, 2014; Rodriguez vd., 2014).

Biyojen aminlerin toksik etkileri

İnsan sağlığı açısından yararlı olan pek çok kritik fonksiyonun yerine getirilmesinde farklı yapıdaki birçok biyojen amine ihtiyaç duyulmasına karşın, yüksek miktarlarda biyojen amin içeren gıdaların tüketimi sonucu bazı toksik etkiler de görülebilmektedir (Guo vd., 2015). Ayrıca alkol ve asetaldehit gibi diğer bileşikler de biyojen aminlerin bağırsak çeperinden geçişini arttırdığından, biyojen aminlerin toksik etkilerini arttırabilmektedirler (Ruız-Capıllas ve Jıménez-Colmenero, 2005; Stadnik ve Dolatowski, 2010). Histamin ve tiramin en toksik ve gıda güvenliği açısından en önemli biyojen aminler olarak kabul edilmektedirler (EFSA, 2011). Yüksek miktarda biyojen amin içeren gıdaların alınması ile histamin ve tiramin toksinlerinin neden olduğu, histamin zehirlenmesi (Scombroid zehirlenme) ve tiramin zehirlenmesi (peynir reaksiyonu) olarak bilinen zehirlenmelere neden olduğu gibi, Tablo 1'de belirtilen çeşitli farmakolojik (vücutta meydana gelen etkiler) etkilerin de ortaya çıkmasına neden olmaktadırlar (Smith, 1980; Stratton vd., 1991).

Histamin zehirlenmesi

Histamin balık, peynir, et ve ürünleri gibi gıdalarda belirlenen ve bir kere oluştuktan sonra ısı etkisiyle dekompoze edilemeyen, kardiyovasküler sistem ve çeşitli salgı bezlerinin hücresel membranlarında bulunan reseptörlere bağlanarak Tablo 1'de belirtilen toksik etkileri gösteren en toksik etkili biyojen amindir (Chong vd., 2011; Çolak ve Aksu, 2002; Rodriguez vd., 2014).

Histamin zehirlenmesine genellikle *Scombridae* ve *Scomberesocideae* familyasına ait (uskumru, ton balığı, torik vb.) balıklar neden olmakla birlikte, bu familyaya ait olmayan (sardalya, hamsi, ringa gibi) balıklar ve ayrıca bazı peynir çeşitleri (Gouda, Swiss, Cheddar, Gruyere vb. peynirlerinde olduğu gibi) de histamin zehirlenmelerine neden olabilmektedirler (Çolak ve Aksu, 2002; Shalaby,1996; Taylor, 1985; Taylor, 1986). Fermente et ürünlerindeki histamin düzeyi de eğer yüksek miktarda tüketilirse toksik etki oluşturabilecek seviyelere ulaşmaktadır (Stratton vd., 1991).

Literatürde, farklı balık çeşitleri için farklı sıcaklıklarda yapılan depolamalar sonucu histamin miktarının değişimi ile ilgili çeşitli çalışmalar bulunmaktadır. Chong vd. (2014) yaptıkları çalışmada Hint uskumru balığını buzda (0°C) ve oda sıcaklığında (25-29°C) depolamış ve histamin miktarının değişimini belirlemişlerdir. Yapılan çalışmada; taze balıkta histamin tespit edilemediği, oda sıcaklığında 20 saatlik depolama sonucunda ise örneklerde 961.64 ppm histamin belirlendiği, buzda 16 günlük depolama sonucunda ise 8.31 ppm histamin bulunduğu ifade edilmektedir.

Tablo 1. Gıdalardaki biyojen aminler ve farmakolojik etkileri (Alvarez ve Moreno-Arribas, 2014; Halász vd., 1994; Shalaby, 1996; Smith, 1980; Velíšek, 2014).

Table 1. Biogenic amines in the foods and their pharmacological effects

Amin	Öncüsü	Diğer Ürünleri	Farmakolojik Etkileri
Histamin (Alifatik, Diamin)	Histidin		Adrenalin ve noradrenalin salınımı. Rahim, bağırsak ve solunum sistemi düz kaslarının uyarılması. Duyusal ve motor nöronların uyarılması. Mide salgısının kontrol edilmesi. Kan basıncının azaltılması. Anafilaktik şok ve alerjik reaksiyonların görülmesi.
Tiramin (Aromatik, Monoamin)	Tirozin	Dopamin, epinefrin, norepinefrin, sinefrin, hordenin	Dopamin öncü maddesi. Kan basıncının arttırılması. Kalp atışının hızlandırılması. Gözyaşı salgılanması. Solunum hızının arttırılması. Kan şekeri seviyesinin arttırılması. Sempatik sinir sisteminden noradrenalin salgılanması. Migren oluşumuna yol açma.
Putresin ve Kadaverin (Alifatik, Diamin)	Ornitin ve lisin	N-metilputresin, spermidin, spermin	Hipotansiyon. Düşük nabız. Tetanoz. El ve ayaklarda kısmi felç. Diğer aminlerin toksisitesinin arttırılması. Makromoleküllerin (nükleik asitlerin) stabilizasyonu, hücre bölünmesinin uyarılması, bitkisel hormon.
β-feniletilamin (Aromatik, Monoamin)	Feniletilamin	Tiramin, dopamin, epinefrin, norepinefrin	Sempatik sinir sisteminden noradrenalin salgılanması. Kan basıncının arttırılması. Migrene neden olma. Memeli sinir sisteminde nörotransmitter. Sinir sistemi düzenleyicisi.
Triptamin (Heterosiklik, Diamin)	Triptofan	Serotonin, melatonin	Kan basıncının arttırılması.
Agmatin (Alifatik, Poliamin)	Arjinin	Putresin, N-metilputresin, spermidin, spermin	Makromoleküllerin (nükleik asitlerin) stabilizasyonu, hücre bölünmesinin uyarılması, bitkisel hormon.
Dopamin (Aromatik, Monoamin)	Dopa	Norepinefrin, epinefrin	Beyinde nörotransmitter madde.

Jinadasa vd. (2015) sarı yüzgeçli orkinos balığını 0°C, 4°C ve 7°C'de 21 gün boyunca depolayıp, balıklardaki histamin miktarının değişimini incelemişlerdir. Yapılan çalışmada; depolamanın başlangıcında balıkta bulunan histamin miktarı 11 ±3 mg/kg olarak belirlenirken, depolama sonunda 0°C, 4°C ve 7°C'de depolama için sırasıyla 21±15, 59 ±44 ve 136 ±25 mg/kg olarak belirlenmiştir. Balıkların farklı sıcaklıklarda depolanması ile ilgili yapılan çalışmalarda araştırmacılar çok farklı sonuçlar kaydetmişlerdir. Bu durum araştırmacılar tarafından balıklarda bulunan mikroorganizma çeşitlerinin ve miktarlarının farklı olmasıyla açıklanmaktadır (Visciano vd., 2012).

El-Kosi vd. (2009) tarafından yapılan bir çalışmada da, Ras peyniri (Mısır'da olgunlaştırılarak üretilen bir peynir çeşidi) 7°C, 22°C ve 32°C'de 120 gün boyunca depolanmış ve depolama boyunca örneklerdeki histamin miktarındaki değişimler saptanmıştır. Yapılan bu çalışmada, başlangıçta 1.73 mg/100 g olan histamin miktarının, 120 günlük depolama sonucunda 7°C, 22°C ve 32°C için sırasıyla 32.57, 76.70 ve 175.30 mg/100g düzeyine çıktığı ifade edilmektedir. Yapılan çeşitli çalışmalar göz önünde bulundurulduğunda histamin oluşumunun, sıcaklık ve depolama süresinin artmasıyla artış gösterdiği görülmektedir. Bu nedenle riskli gıdalarda bu faktörler göz önünde bulundurularak üretim yapılmalıdır.

Histamin düşük seviyedeyken tek başına zehirlenmeye neden olmamakla birlikte ortamda putresin ve kadaverin gibi diğer biyojen aminlerin olması; diamino oksidaz (DAO) enzimlerini inhibe etmekte, histaminin bağırsaklardan geçişini

arttırmakta ve böylece histaminin toksik etkisini 5 kat arttırmaktadır (Emborg ve Dalgaard, 2008; Hernández-Jover vd., 1997; Rodriguez vd., 2014; Taylor ve Speckhard, 1983). Sağlıklı insanlar diyetteki histamini hızlıca metabolize edebilmektedirler fakat histaminin birikmesiyle histamin detoksifikasyon kapasitesi aşılabilmekte ve böylece histamin toksisitesi görülebilmektedir. Alkol, antihipertensif, antidepresan, antihipotonikler, antiaritrik ilaçlar ve diamino oksidaz enzimini inhibe eden diğer ilaçları kullanan kişilerde histamin intoksikasyonu şüphesinin arttığı belirtilmektedir (Chong vd., 2011).

Tiramin zehirlenmesi

Peynir reaksiyonu olarak da adlandırılan tiramin zehirlenmesinin; peynir tüketiminin, monoamino oksidaz inhibitörü (MAOI) ilaçlarla tedavi gören hastalarda hipertensif rahatsızlıklara yol açan başlıca gıda olduğu bildirilmektedir (Spano vd., 2010). Bu hastalığın peynir reaksiyonu olarak adlandırılmasının temel nedeni, toksik vakaların çoğunun peynir tüketimine bağlı olarak oluşmasıdır fakat sadece peynir değil aynı zamanda maya ekstraktları, salamura ringa balığı, kuru sosis, alkollü içecekler, çoğu baklagiller, tavuk karaciğeri, bira gibi gıdaların da bu reaksiyona neden olabilecekleri belirtilmektedir. Yüksek miktarda tiramin içeren gıdalar tüketildiğinde, metabolize olamayan yüksek miktarda tiramin kana geçmekte ve bu da sempatik sinir sisteminden noradrenalinin salınmasına neden olmakta, böylelikle çevresel damarların büzülmesi ve kalp atışlarının hızlanmasıyla kan basıncı artmaktadır. Yüksek miktarda tiramin içeren gıdaların tüketilmesi sonucu monoamino oksidaz enzimini inhibe eden maddeleri (çoğu kez ilaç kaynaklı) vücutlarında bulunduran bireylerde Tablo1'de belirtilen toksik etkiler ortaya çıkabilmektedir (Glória, 2005; Smith, 1980; Shalaby, 1996).

Budak vd. (2008) tarafından yapılan çalışmada, beyaz peynirin 4°C, 10°C ve 20°C'de 90 gün depolanması sonucunda tiramin miktarında depolama boyunca değişme gözlenmediği, depolama başlangıcı ve sonunda peynir örneklerinde tiraminin tespit edilemediği belirtilmiştir. El-Kosi vd. (2009) tarafından yapılan çalışmada ise Ras peyniri 7°C, 22°C ve 32°C'de 120 gün boyunca depolanmış ve tiramin miktarındaki değişimler belirlenmiştir. Yapılan çalışmada, başlangıçta 0.03 mg/100 g olan tiramin miktarının, 120 günlük depolama sonucunda 7°C, 22°C ve 32°C için sırasıyla 1.69, 3.26 and 10.95 mg/100g düzeyine ifade edilmiştir.

Diğer biyojen aminler

Diğer biyojen aminlerden spermin ve spermidin gibi vücut fizyolojisi açısından yararlı olduğu belirlenmiş poliaminlerin alınan miktara göre, gıda alerjisiyle ilişkili olabildikleri belirtilmektedir (Ruız-Capıllas ve Jıménez-Colmenero, 2005). Ayrıca spermin, spermidin ve putresin büyüme ve gelişmede önemli rol oynadıklarından katabolize edilmeleri sınırlıdır böylelikle tümör gelişimini hızlandırabilmektedirler, bundan dolayı kanser tedavisi araştırmalarının en önemli hedeflerinden birinin de kanserli hastalarda poliaminlerin inhibe edilmesinin olduğu ifade edilmektedir (Bardócz, 1995; Rodriguez vd., 2014). Poliaminlerin doğrudan kanseri tetiklemedikleri, buna karşın tümör gelişimini hızlandırdıkları; vücutta poliamin miktarının artmasıyla hücre bölünmesinin arttığı, çevredeki bağışıklık hücrelerinin antitümör bağışıklık fonksiyonlarını kaybettiği ve böylelikle kanser hücrelerinin istila etme ve yayılma yeteneklerinin arttığı belirtilmektedir (Soda, 2011).

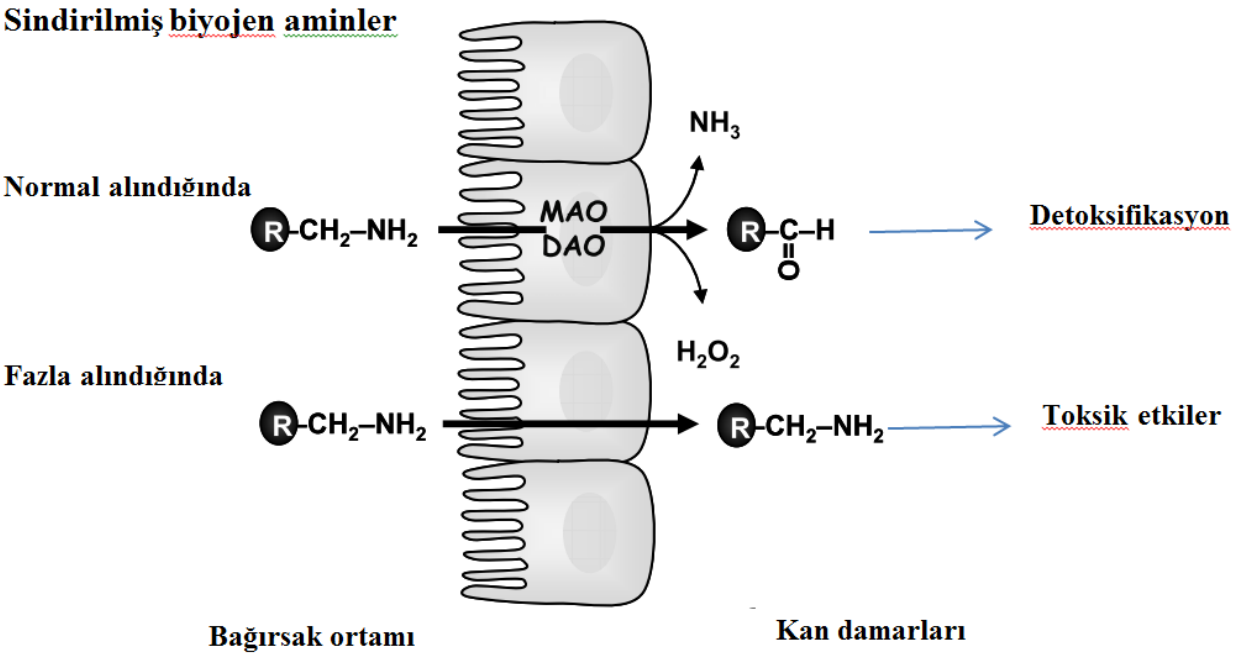
Putresin ve kadaverin gibi alifatik aminler ve triptamin ve β- feniletilamin gibi heterosiklik aminler, aromatik aminlere göre daha az toksik etki göstermelerine rağmen yüksek miktarlarda alındıklarında Tablo 1'de belirtilen toksik etkileri gösterebilmektedirler. Ayrıca putresin (nitrozopirolidin'e), kadaverin (nitrozopiperidin'e), agmatin, spermin, tiramin ve spermidin gibi sekonder aminler ise, nitrit ile reaksiyona girerek karsinojenik nitrozaminlere dönüşebilmektedirler (Brink vd., 1990; Glória, 2005; Joosten, 1988; Silla-Santos., 1996). Serotonin ise düşük miktarlarda alındığında risk oluşturmazken, genetik bozukluğu olan bireylerde veya monoamino oksidaz inhibitörü (MAOI) ve serotonin sendromuna neden olan diğer ilaçların tedavide eş zamanlı olarak kullanılmasıyla Serotonin Sendromu denilen (nadir olarak görülen ancak ölümcül olabilen) reaksiyona neden olduğu kaynaklarda belirtilmektedir (Özdemir ve Kocabaşoğlu, 2007).

Biyojen Aminlerin Detoksifikasyonu (Katabolize Edilmesi)

Biyojen aminler potansiyel toksik etkilerinden dolayı oldukça önemli bileşiklerdir ancak tüm biyojen aminlerin aynı toksik etkiye sahip olmadıkları bilinmektedir. Histamin, tiramin ve feniletilamin en fazla toksik etki gösteren biyojen aminler olması nedeniyle gıdalarda biyojen aminlerin yasal sınırlandırılmasında daha çok bu aminler ön plana çıkmaktadır. Histamin zehirlenmesine yaygın olarak dünya genelinde rastlanılmakla birlikte, peynir tüketiminin yüksek olduğu ülkelerde, tiraminin tek başına ve yüksek konsantrasyonlarda vücuda alınmasıyla beraber tiramin zehirlenmesi görülebilmektedir. Bu nedenle Almanya ve Hollanda gibi ülkelerde balık ve şarap gibi ürünlerde histamin değeri için üst limit değerleri belirtilmekteyken, ülkemizde ise sadece balıkta histamin konsantrasyonu için bir üst sınır değeri verilmiştir (Özdestan ve Üren, 2012; Silla-Santos., 1996; Shalaby, 1996). Sağlıklı bireylerin gıdalardaki biyojen aminleri

çoğunlukla asetilasyon veya monoamino oksidaz (MAO) ve diamino oksidaz (DAO) enzimlerini kullanarak oksidasyon mekanizması ile katabolize ettikleri, ancak poliaminlerin genellikle asetillendirildikten sonra diamino oksidaz (DAO) ve poliamino oksidaz (PAO) enzimleri tarafından okside edildikleri belirtilmektedir (Glória, 2005; Rodriguez vd., 2014). Biyojen aminler vücuda çok fazla miktarda alınmadıkları sürece insanlarda sağlık riski göstermemekle birlikte alerjisi olan bireylerde, biyojen aminleri katabolize eden enzimlerin inhibe edildiği veya genetik bozukluğun olduğu durumlarda detoksifiye edilemeyip vücutta birikebildikleri ve toksik etki gösterebildikleri ifade edilmektedir (Halász vd., 1994; Karovičová ve Kohajdová, 2005; Rodriguez vd., 2014; Stadnik ve Dolatowski, 2010). Solunum sistemi hastalıkları, koroner kalp hastalıkları ve hipertansiyon problemi olanlar, B_{12} vitamini eksikliği olan bireyler, gastrointestinal problemleri olan (gastrit, tahriş olmuş bağırsak sendromu, Crohn hastalığı, gastrik ve kolon ülseri) bireylerin bağışıklık sistemiyle ilgili problemleri veya bağırsaklarında daha az amino oksidaz enzimi bulundurmaları nedeniyle biyojen aminlere daha duyarlı oldukları ifade edilmektedir (Bardócz, 1995; Rodriguez vd., 2014).

Şekil 1'de gösterilen oksidasyon mekanizması biyojen aminlerin yıkımlanması için temel yol olmasına rağmen, metilasyon ve asetilasyon da histaminin yıkımlanmasında etkili olmaktadır (Glória, 2005; Lehane ve Olley, 2000).



Şekil 1. Biyojen aminlerin bağırsaklarda oksidasyon mekanizması ile katabolize edilmesi (Kantaria ve Gokani, 2011).

Figure 1. Catabolism of biogenic amines by oxidation mechanism in the human intestinal tract

Histamin ve putresin bağırsaklarda DAO enzimleri tarafından deamine edilirken, tiraminin yıkımlanması birkaç katabolitik reaksiyonla gerçekleştirilebilmektedir. Tiramin daha çok gastrointestinal mukoza ve karaciğerde metabolize edilmekte ve tiramin katabolizması için temel yol MAO tarafından gerçekleştirilen p-hidroksifenil asetik aside oksidatif deaminasyondur. Diğer yollar ise dopamin-β-hidroksilaz tarafından oktopamine oksidasyon, N-metil transferaz tarafından N-metil tiramine metilasyon ve sülfat veya asetat grupları ile konjugasyondur (Glória, 2005; McCabe-Sellers vd., 2006).

Genel anlamda memeliler, histaminin % 60-80'ini oksidatif deaminasyon ile katabolize edebilmektedirler buna karşın insan vücudunda histamin, amino oksidazların etkisiyle iki farklı mekanizmayla katabolize edilebilmektedir. Birinci mekanizmada, imidazol çevrimindeki azot, N-metilhistamin oluşturmak üzere histamin N-metiltransferaz (HMT) tarafından metillendirilir daha sonra monoamino oksidaz tarafından N-metil imidazol asetik aside okside edilmekte; ikinci mekanizmada ise histamin, riboza bağlanan diamino oksidaz tarafından imidazol asetik aside okside edilmektedir (Glória, 2005; Stratton vd., 1991). Histamin midede bakteriler tarafından inaktif asetil histamine dönüştürülebilmekte, ayrıca böbrekler de histaminin kandan uzaklaştırılmasında rol oynayabilmektedirler (Karovičová ve Kohajdová, 2005). Histamin toksisitesi için tedavide histamin reseptörlerini bloke ederek histaminin etkilerini inhibe eden antihistamin ilaçlarının (etanolamin, etilendiaminler, alkilaminler, piperazin türevleri, fenotiyazinler ve piperidin türevleri gibi) kullanılması önerilmektedir (Anonim, 2010; Attaran ve Probst, 2002).

DAO enzimi seçici olmayan, putresin ve kadaverin gibi birçok biyojen amin üzerine etkili olabilen bir enzim iken histamin N-metil transferaz (HMT) ise histamine spesifiktir (Taylor ve Sumner, 1986). Bununla beraber DAO enzimini yüksek oranda inhibe eden en potansiyel inhibitör maddelerin amioguanidin (%100), karnosin (%100), histamin (%99), agmatin (%97), tiamin (%92), kadaverin (%87) ve tiramin (%77) olduğu; kafein, hipoksantin, indol, 1-metilhistidin, feniletilamin, piperazin, spermidin, spermin, sinefrin, teobromin, teofilin, triptamin ve ksantinin ise orta düzeyde bu inhibisyona katılabildiği ifade edilmektedir (Alvarez ve Moreno-Arribas, 2014; Taylor ve Lieber, 1979).

Sonuç

Biyojen aminler, vücutta çeşitli fonksiyonların yerine getirilmesinde önemli rol oynayan ve yaşam için elzem bileşikler olarak karşımıza çıkmaktadır. Gıdalarda biyojen aminler çoğunlukla dekarboksilaz pozitif etki gösteren mikroorganizmaların gelişmesi sonucu oluştuğundan bu maddeler aynı zamanda gıdaların tazelikleri ve kalitesinin belirlenmesinde indikatör olarak da kullanılabilmektedirler. Bununla birlikte bazı biyojen aminler vücuda fazla miktarda alındıklarında, vücut tarafından detoksifiye edilemedikleri için toksik etkiler gösterebilmektedirler. Bu nedenle gıdalarda bulunan biyojen aminlerin konsantrasyonlarının belirlenmesi ve bu aminlere duyarlı bireylerde diyetle alınan amin miktarının kontrol altına alınması önem taşımaktadır. Biyojen aminlerden histamin ve tiramin toksisitesine daha sık rastlanıldığından, bu biyojen aminlerle ilgili çalışmalar daha fazla sayıda iken, daha sonra tanımlanmış (agmatin gibi) diğer biyojen aminlerin insan sağlığı açısından değerlendirilerek bunların etkilerinin belirlenmesi üzerine çeşitli çalışmaların yapılması ve potansiyel toksisite gösteren diğer biyojen aminler için de gıdalarda bulunması gereken üst limitleri üzerine yasal düzenlemelerin oluşturulması önem arz etmektedir.

Kaynaklar

Alper, N., Temiz, A. (2001). Gıdalardaki biyojen aminler ve önemi. *Türk Hijyen ve Deneysel Biyoloji Dergisi,* 58(2), 71-80.

Alvarez, M.A., Moreno-Arribas, M.V. (2014). The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in Food Science and Technology*, 39(2), 146-155.

Anonim (2010). Anestezi ve reaminasyon. Premedikasyon ilaçları, Ankara. http://www.megep.meb.gov.tr/mte_program_modul/moduller_pdf/Premedikasyon%20%C4%B0la%C3%A7lar%C4%B1.pdf (Erişim Tarihi: 20.07.2016)

Askar, A., Treptow, H. (1986). Biogene amine in Lebensmitteln. Vorkommen, Bedeutung und Bestimmung, Eugen Ulmer GmbH and Co, Stuttgart, Germany. ISBN: 9783800121328

Attaran, R.R., Probst, F. (2002). Histamine fish poisoning: A common but frequently misdiagnosed condition. *Emergency Medicine Journal*, 19(5), 474-475.

Azim, Ö. (2002). Gıdalarda yüksek basınç sıvı kromatografisi (HPLC) ile biyojen amin analizleri, Yüksek Lisans Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü, 89 s.

Bardócz, S. (1995). Polyamines in food and their consequences for food quality and human health. *Trends in Food Science and Technology*, 6(10), 341-346.

Budak, H.N.F., Karahan, A.G., Çakmakçı, M.L. (2008). Factors affecting histamine and tyramine formation in Turkish white cheese. *Hacettepe Journal of Biology and Chemistry*, 36(3), 197-206.

Brink, B. ten, Damink, C., Joosten H.M., Huis in't Veld, J.H. (1990). Occurrence and formation of biologically active amines in foods. *International Journal of Food Microbiology*, 11(1), 73-84.

Cardozo, M., Souza, S.P. De, Lima, K. S. C., S.Lima, A.L. (2011). Degradation of biogenic amines by gamma radiation process and identification by GC/MS, International Nuclear Atlantic Conference, Brazil, 24-28.

Chong, C.Y., Abu Bakar F., Russly A.R., Jamilah, B., & Mahyuddin, N.A. (2011). The effects of food processing on biogenic amines formation. *International Food Research Journal*, 18(3), 867-876.

Chong, C.Y., Abu Bakar, F., Rahman, R.A., Bakar, J., Zaman, M.Z. (2014). Biogenic amines, amino acids and microflora changes in Indian mackerel (*Rastrellinger kanagurta*) stored at ambient (25-29°C) and ice temperature (0 °C). *Journal of Food Science and Technology*, 51(6), 1118-1125.

Çolak, H., Aksu, H. (2002). Gıdalarda biyojen aminlerin varlığı ve amin oluşumunu etkileyen faktörler. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 13(1-2), 35-40.

EFSA. (2011). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2393.pdf

El-Kosi, O.H.R., Abdel-Hakiem E.H., Ayesh, A.M., Mohamed, J.I.I. (2009). Effect of different storage temperatures and periods on biogenic amines formation in Ras Cheese. *Suez Canal Veterinary Medicine Journal, VIX*(1), 207-218.

Emborg, J., Dalgaard, P. (2008). Modelling the effect of temperature, carbon dioxide, water activity and pH on growth and histamine formation by *Morganella psychrotolerans*. *International Journal of Food Microbiology*, 128(2), 226-233.

Flick, G.J., Granata, L.A. (2005), Biogenic amines in foods, W.M. Dabrowski and Z.E. Sikorski, (Eds.), Toxins in Food, CRC Press, Boca Raton, pp. 121-154. ISBN:9780849319044

Glória, M.B.A. (2005). Bioactive amines, H. Hui and L.L.Nollet, (Eds.), Handbook of Food Science, Technology and Engineering, Taylor & Francis, New York, Vol.1, pp. 13-32. ISBN:9781420027518

Guo, Y.Y., Yang, Y.P., Peng, Q., & Han, Ye. (2015). Biogenic amines in wine: A review. *International Journal of Food Science and Technology, 50*(7), 1523-1532.

Gürbüz, O. (2002). Şarapta biyojen aminler. *Gıda*, *27*(2), 85-91.

Halász, A., Baráth, A., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology*, 5(2), 42-49.

Hernández-Jover, T., Izquiermo-Pulido, M., Veciana- Nogués, M. T., Mariné-Font, A., VidaL-Carou, M.C. (1997). Biogenic amine and polyamine contents in meat and meat products. *Journal of Agricultural and Food Chemistry*, 45(6), 2098-2102.

Jinadasa, B.K.K.K., Galhena, C.K., Liyanage, N.P.P. (2015). Histamine formation and the freshness of yellowfin tuna (*Thunnus albacares*) stored at different temperatures. *Cogent Food and Agriculture*, 1(1028735), 1-10.

Joosten, H.M.L.J. (1988). The biogenic amine contents of Dutch cheese and their toxicological significance. *Netherlands Milk and Dairy Journal*, 42(1), 25-42.

Kalač, P. (2014). Health effects and occurrence of dietary polyamines: A review for the period 2005-mid 2013. *Food Chemistry*, 161, 27-39.

Kantaria, U.D., Gokani, R.H. (2011). Quality and safety of biogenic amines. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(4), 1461-1468.

Karovičová, J., Kohajdová, Z. (2005). Biogenic amines in food. *Chemical Papers*, 59(1), 70-79.

Lee, Y.H., Kim, B.H., Kim, J.H., Yoon, W.S., Bang, S.H., Park, Y.K. (2007). CadC has a global translational effect during acid adaptation in *Salmonella enterica serovar typhimurium*. *Journal of Bacteriology*, 189(6), 2417-2425.

Lehane, L., Olley, J. (2000). Histamine fish poisoning revisited. *International Journal of Food Microbiology*, 58(1-2), 1-37.

McCabe-Sellers, B.J, Staggs, C.G., Bogle, M.L. (2006). Tyramine in foods and monoamine oxidase inhibitor drugs: A crossroad where medicine, nutrition, pharmacy, and food industry converge. *Journal of Food Compostion and Analysis*, 19, 58-65.

Mohamed, A.R., Toliba, A.O. (2013). Effect of irradiation and storage on biogenic amine contents in ripened Egyptian smoked cooked sausage. *Journal of Food Science and Technology*, 50(6), 1165- 1171.

Naila, A., Flint, S., Fletcher, G., Bremer, P., Meerdink, G. (2010). Control of biogenic amines in food-existing and emerging approaches. *Journal of Food Science*, 75(7), 139-150.

Önal, A. (2007). A review : Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*, 103(4), 1475-1486.

Özdemir, S., Kocabaşoğlu, N. (2007). Serotonin sendromuna güncel bir yaklaşım. *Klinik Psikofarmakoloji Bülteni,* 17(4), 217-225.

Özdestan, Ö., Üren, A. (2012). Gıdalarda biyojen aminlerle ilgili yasal düzenlemeler. *Gıda ve Yem Bilimi-Teknolojisi Dergisi*, 12, 27-40.

Özoğul, F., Küley, E., & Özoğul, Y. (2004). Balık ve balık ürünlerinde oluşan biyojenik aminler. *Ege Ürünleri Su Ürünleri Dergisi,* 21(3-4), 375-381.

Rhee, J.E., Rhee, J.H., Ryu, P.Y., Choi, S.H. (2002). Identification of the cadBA operon from *Vibrio vulnificus* and its influence on survival to acid stress. *FEMS Microbiology Letter*s, 208(2), 245–251.

"Rodriguez, M., Carneiro, C., Feijó, M., Júnior, C., Mano, S. (2014). Bioactive amines: Aspects of quality and safety in food. *Food and Nutrition Science*, 5(2), 138-146.

Ruiz-Capillas, C., Jiménez-Colmenero, F. (2005). Biogenic amines in meat and meat products. *Critical Reviews in Food Science and Nutrition*, 44(7-8), 489-599.

Shalaby, A.R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International*, 29(7), 675-690.

Silla-Santos, M. H. (1996). Biogenic amines: their importance in foods. *International Journal of Food Microbiology*, 29(2-3), 213-231.

Smith, T. A. (1980). Amines in food. *Food Chemistry*, 6(3), 169-200.

Soda, K. (2011). The mechanisms by which polyamines accelerate tumor spread. *Journal of Experimental and Clinical Cancer Research*, 30(95), 1-9.

Spano, G., Russo, P., Lonvaud-Funel, A., Lucas, P., Alexandre, H., Grandvalet, C., Coton, E., Coton, M., Barnavon, L., Bach, B., Rattray, F., Bunte, A., Magni, C., Ladero,V., Alvarez, M., Fernández, M., Lopez, P., de Palencia, P.F., Corbi, A., Trip, H., Lolkema, J.S. (2010). Biogenic amines in fermented foods. *European Journal of Clinical Nutrition*, 64(3), 95-100.

Stadnik, J., Dolatowski, Z. J. (2010). Biogenic amines in meat and fermented meat products. *Acta Scientiarum Polonorum Technologia Alimentari*, 9(3), 251-263.

Stratton, J.E., Hutkins, R.W., Taylor, S.L. (1991). Biogenic amines in cheese and other fermented food: A review. *Journal of Food Protection*, 11(54), 460-470.

Tassoni, A., Germana, M.A., Bagni, N. (2004). Free and conjugated polyamine content in *Citrus sinensis* Osbeck, cultivar Brasiliano N.L. 92, a Navel orange at different maturation stages. *Food Chemistry*, 87(4), 537-541.

Taylor, S. L. (1985). Histamine poisoning associated with fish, cheese, and other foods, World Health Organization, Geneva. http://apps.who.int/iris/handle/10665/66407

Taylor, S.L. (1986). Histamine food poisoning: toxicology and clinical aspects. *Critical Reviews in Food Toxicology*, 17(2), 91-128.

Taylor, S.L., Lieber, E.R. (1979). In vitro inhibition of rat intestinal histamine-metabolizing enzymes. *Food and Cosmetics Toxicology*, 17(3), 237-240.

Taylor, S.L., Speckhard, M.W. (1983). Isolation of histamine-producing bacteria from frozen tuna. *Marine Fisheries Review*, 45(4-6), 35-39.

Taylor, S.L., Sumner S.S. (1987). Determination of histamine, putrescine and cadaverine. D.E. Kra-mer and J.Liston (Eds). Seafood Quality Determi-nation. Elsevier, New York, pp. 235-242. ISBN: 9780444428950

Til, H.P., Falke, H.E., Prinsen, M.K., Willems, M.I. (1997). Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. *Food and Chemical Toxicology*, *35*(3-4), 337–348.

Uylaşer, V., Konak, A. (2004). Gıdalardaki biyojen aminler ve insan sağlığı açısından önemi. *Gıda ve Yem Bilimi-Teknolojisi*, 6, 26-33.

Velíšek, J. (2014). The chemistry of food, Hoboken, NJ: Wiley-Blackwell, pp. 818-827. ISBN: 9781118383810

Visciano, P., Schirone, M., Tofalo, R., Suzzi, G. (2012). Biogenic amines in raw and processed seafood. *Frontiers in Microbiology*, *3*(188), 1-10.

Yerlikaya, P., Gökoğlu, N. (2002). Gıdalarda biyojen aminler ve önemi. *Gıda Mühendisliği Dergisi*, 6(12), 24-30.

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SIMULTANEOUS DETERMINATION OF BENZOIC ACID AND SORBIC ACID IN FOOD PRODUCTS BY CAPILLARY ELECTROPHORESIS

Nevin Öztekin

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Istanbul Technical University, Department of Chemistry, Maslak, 34469 Istanbul, Turkey

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Correspondence:

Nevin ÖZTEKİN

E-mail: noztekin@itu.edu.tr

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ABSTRACT

In this work, a rapid and easy capillary electrophoretic method for simultaneous determination of benzoic acid and sorbic acid in food samples using direct UV detection was reported. The separation of acids was achieved in fused silica capillary at 28 kV. 20 mM borate buffer at pH 9.3 was used as running buffer; cinnamic acid was chosen as internal standard for quantification. Under the optimal separation conditions benzoic acid and sorbic acid were detected in 3.1 min. The linear ranges were between 0.005-0.4 mM. The correlation coefficients for each calibration curve were calculated as 0.999. The reproducibility of peak area and migration time for each acid were less than 3% (R.S.D.). The limits of detection were found 0.405 µg/mL for benzoic acid and 0.415 µg/mL for sorbic acid. The limits of quantification were 1.35 µg/mL and 1.38 µg/mL respectively.

Keywords: Capillary electrophoresis, Benzoic acid, Sorbic acid, Foods

Introduction

Benzoic acid (E210) and sorbic acid (E200) are widely used as food additives to delay the microbiological contamination due to action of bacteria, yeasts and moulds in various foods. They are supposed as usually reliable preservatives. If food products contain high concentrations of these acids, they may damage to health of consumers due to causing allergic reactions. Like the other countries, the use of these additives have been restricted according to Turkish Food Codex regulations of Agriculture Ministry of Turkey. In order to protect human health, determination of maximum permitted amounts of these additives in food products is very important. Consequently, easy and reliable analysis methods for detection of these additives in foodstuffs is required for food safety. Several analytical methods are available for the determination of additives in foods like UV spectrometry (Marsili, et al., 2003; Naseri, et al. 2017) and GC (Ochiai et al., 2002; Abedi et al. 2014). Both of these methods require comprehensive sample preparation and relatively long analysis time. HPLC is most commonly used method for the determination of these additives in foods and beverages (Mota et al., 2003; Pylypiw Jr & Grether, 2000; Tfouni & Toledo, 2002; Zor et al. 2016; Petanovska-Ilievska et al. 2017). HPLC generally needs the consumption of large amounts of organic solvents during the analysis and extensive sample preparation step, including solid phase extraction.

Capillary electrophoresis (CE) is an analytical method that offer high separation efficiency and is ensuring a fast separation for the assay of several species. CE method has some advantages such as fast analysis time, low or no organic solvent consumption and little injection volume. A matrix effect in sample can be eliminated with short and simple sample pretreatment. The capillary between runs is quickly washed with running buffer. Therefore it is a convenient analysis technique for using in the analysis of foods contained complex matrices.

The inhibitory action of weak acids is classically believed to be due to the undissociated compounds. Because, the uncharged, undissociated state of the acid is freely passed through the plasma membrane and is thus ensured to enter the cell (Brul & Coote, 1999). pK_a values of benzoic and sorbic acid are smaller than pH 7 (4.19 and 4.76 respectively). The activity of them in foods that has value of low pH increase. These acids are used as a substitute for each other. Nevertheless, we observed the mixtures of benzoic and sorbic acid are used in some food products in Turkey. While several of the CE reports demonstrated the possibilities for simultaneous determination of benzoic and sorbic acids (Boyce, 1999; Frazier et al., 2000; Kuo & Hsieh, 1997; Lin et al., 2000; Pant & Trenerry, 1995; Waldron & Li, 1996; Han et al., 2008; Hsu et al., 2014; Sun et al., 2014; Li et al., 2015; Aung & Pyell 2016), only a few of them reported benzoic and sorbic acids together in real samples.

This study presents an application for the quantification of benzoic and sorbic acid in the food samples in the Turkish markets by using CE method. Before the injection processes, any pretreatment procedure was not carried out for the food samples.

Materials and Methods

Instrumentation

For separations and determinations, an Agilent CE system (Waldbronn, Germany) equipped with a diode array detector was used. Agilent Chem Station software performed all system control and data processing. In separations, 50µm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with 46 cm total length and 38 cm effective length was used. The capillary temperature was set at 25ºC. The separations were performed at voltage of 28 kV. The injection of samples was made at pressure 50 mbar for 4s. Before injections at the beginning of every day the capillary was conditioned with 0.1 M NaOH solution for 15 min, deionized water for 5 min and running buffer for 10 min. between runs 1 min of flushing with running buffer was performed. A Metrohm 654 Digital pH Meter was used for pH measurements. All solutions were prepared with deionized water obtained from an Elgacan C114 filtration system.

Chemicals

Benzoic acid, sorbic acid, and cinnamic acid were purchased from Fluka (Fluka AG, Buchs, Switzerland). Sodium borate was obtained from Merck (Darmstadt, Germany). Other chemicals used were of analytical reagent grade. The food samples were from local market.

Standards

Stock solutions of benzoic acid, sorbic acid, and cinnamic acid were prepared by dissolving in water, and stored at 4ºC.

Samples

After solid samples were homogenized, 1-2 g samples were weighed in a beaker. 15 mL of deionized water was added to samples and stirred during 30 min. The volume of sample was diluted to 20 mL. The solutions were centrifuged a 7000 rpm for 5 min and filtered through a 0.45 µm syringe filter. Internal standard solution (cinnamic acid) was added to filtrate and the sample solution volume was diluted with water. The completed solution was injected directly into the capillary. Liquid food samples were prepared by dilution with water.

Results and Discussion

In CE analysis the electroosmotic flow (EOF) plays an important role. The magnitude of the EOF can influence the resolution and separation efficiency. As we all know, the EOF can be determined by net surface charge of the inner surface of capillary. At higher pH (usually alkaline), faster EOF are obtained due to the full ionization of silanol groups. The concentration and pH of buffer are the two important parameters for adjusting the EOF and the electrophoretic mobility. Therefore the separation buffer affects the migration time and the resolution between analytes. The ionization degree of an organic acid also depends on choice of the buffer. At the same time the pH of the buffer influences ionization degree and separation of organic acids. Simultaneous migrations of benzoic acid, sorbic acid, and cinnamic acid were tested in the three different separation buffer namely; borate, phosphate and Tris in the pH range of 7 and 9.5. Their pK_a values are 4.19, 4.76 and 4.27, respectively and in this pH range all acids are in higher ionization degrees. Thus they can be separated as anions in alkaline media.

When borate buffer was used as the separation media, peak heights and peak areas were observed as better than the peaks in the other two buffers, and also migration times were shorter. Therefore borate was selected as the running buffer. Dilute buffer solutions are desirable to shorten migration time. However, it is important that buffer solutions have sufficient buffer capacity to maintain a fixed pH. Inadequate buffer capacity may result in the poor resolution. The effect of borate concentration was studied within the range 10-50 mM. At higher borate concentrations (>50 mM) longer migration time and broader peak were obtained. Optimum peak symmetry and peak area were achieved with 20 mM borate.

When the running voltages is increased the efficiency of the separation improves and the migration time shortens. In the higher running voltage, the efficiency of the separation decrease due to the Joule heating. On the other hand, the reproducibility and the resolutions between acids becomes worse if the running voltage decreases. In this study a voltage of 28 kV was selected as the best separation voltage with acceptable compromise between adequate resolution and short migration time.

In CE the high amounts of matrix components in the samples affect the peak shapes of analytes has generally low concentrations. In order to check the matrix effect on the peak shapes, the real samples was injected to the borate buffer with increasing concentrations. It was not observed a distortion in the peak shapes in the small borate concentrations. Since the matrix effect was not observed in the highly diluted real samples, all separations were performed in the 20 mM borate buffer at pH 9.3, considering the fast separation times.

Figure 1 shows the absorption spectra of the analytes and internal standard in the same concentration level. In Figure 2 the peak areas of benzoic acid, sorbic acid, and cinnamic acid were given at the different wavelengths varying from 210 to 270 nm. According to peak areas 220 nm can be selected for the simultaneous screening of benzoic acid, sorbic acid, and cinnamic acid peaks. In this study because of using a diode array detector for the detection, benzoic acid and sobic acid was dedected different wavelengths respectivly 220 nm and 260 nm.

The electropherogram of standard mixture of acids at optimum experimental conditions are shown in Figure 3a. The samples were injected directly to the borate buffer and all acids were detected in about 3.0 min.

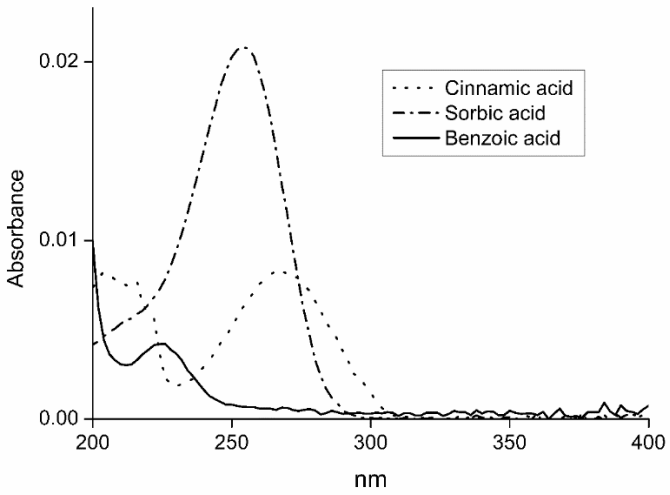

Figure 1. Spectra of benzoic, sorbic and cinnamic acid

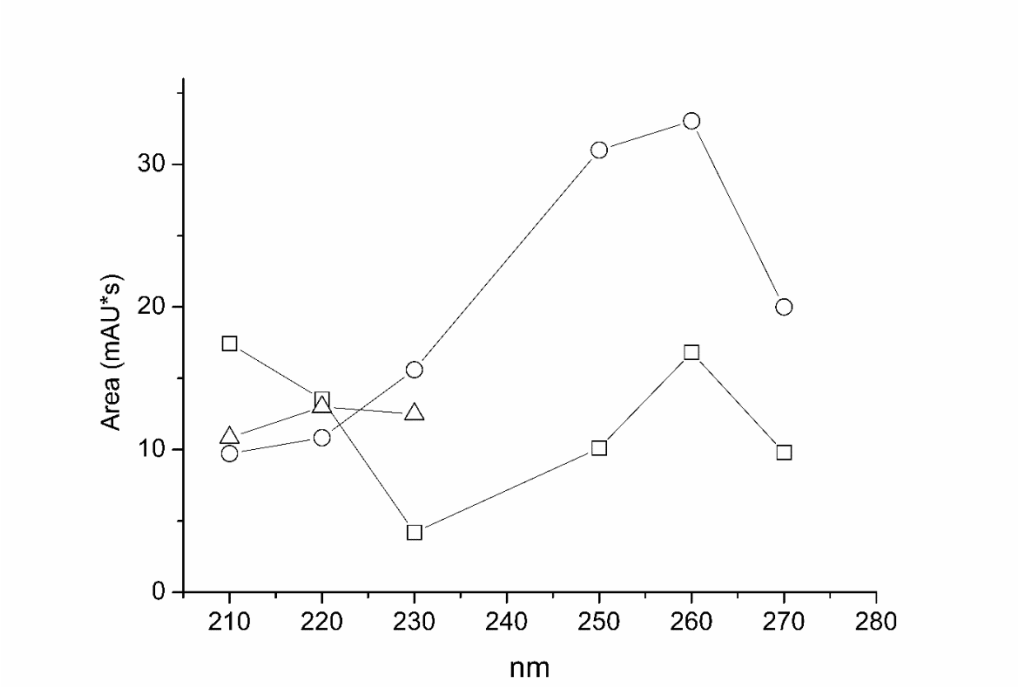

Figure 2. The effect of the wavelength on the peak areas of benzoic acid (△), sorbic acid (○) and cinnamic acid (□). Running buffer, 20 mM Borate, pH 9.3; injection, 4 s at 50 mbar; run voltage, 28 kV

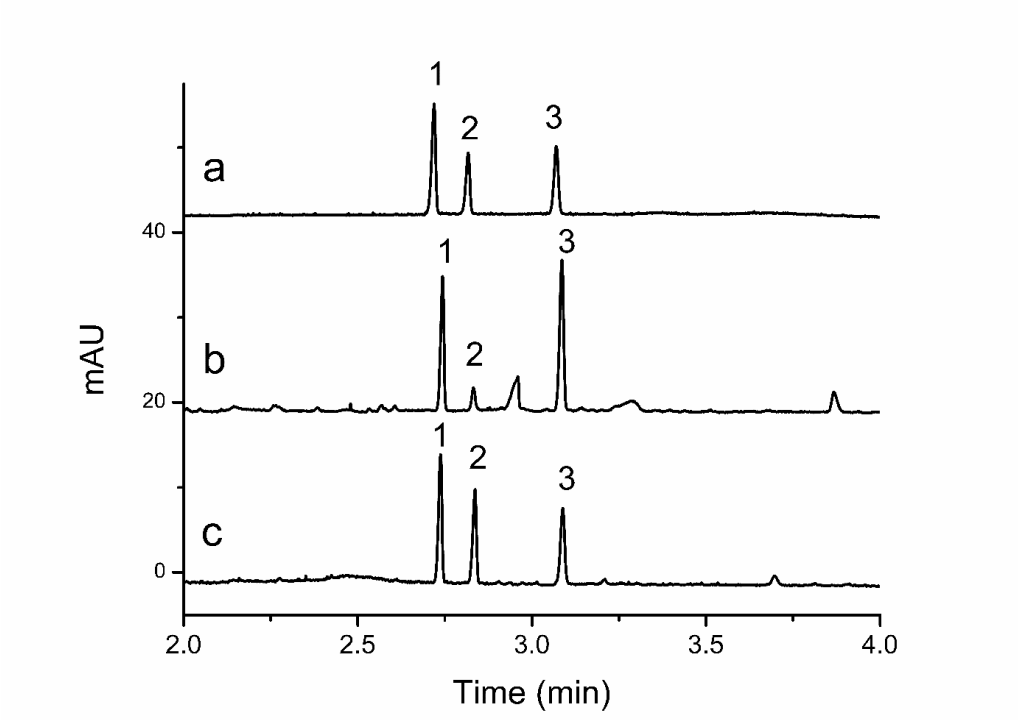

Figure 3. Electropherograms of acids. (a) standard solution; (b) ketchup sample and (c) chestnut sweet sample. Peaks: (1) cinnamic acid, (2) sorbic acid, (3) benzoic acid. Experimental conditions: capillary, fused-silica 50 μm i.d. and 46 cm (38 cm effective length); background electrolyte, 20 mM Borate, pH 9.3; injection, 4 s at 50 mbar; temperature 25^{0}C; running voltage 28 kV; detection signal = 220 nm

Method Validation

Linearity

The calibration curves were obtained between the peak area ratios ($A_{analyte}/A_{internal\ standard}$) and the concentrations of the acids. The calibration curves for benzoic acid and sorbic acid showed linear ranges between 0.005 and 0.4 mM. Linear regression equations for benzoic acid and sorbic acid were obtained as $y = 8.140\ 10^{-1}\ x - 9.558\ 10^{-4}$ and $y = 6.474\ 10^{-1}\ x - 3.459\ 10^{-3}$ respectively. The correlation coefficients values were greater than 0.999.

Limit of detection

Limits of detection (LOD) were calculated as three times the baseline noise (S/N=3). The LOD value was 0.405 µg/mL for benzoic acid and 0.415 µg/mL for sorbic acid. Limits of quantification (LOQ) (S/N=10) was 1.35 µg/mL and 1.38 µg/mL respectively.

Reproducibility

The reproducibility of the CE method was determined by analyzing two real samples, Ketchup and Chestnut candy. % RSD values of migration times and peak areas were calculated for intra-day and inter-day. Intra-day and inter-day precision were calculated seven successive injections in a day and three injections in different days, respectively. The results are given in Table 1.

Table 1. Reproducibilities of the method for two sample. (I) Ketchup, (II) Chestnut sweet

	Intraday RSD%		**Inter day RSD%**		**mg anion/kg sample RSD %**	
	Migration time	**Peak area**	**Migration time**	**Peak area**	**Inter day**	**Intra day**
			Sample 1			
Benzoic	0.213	0.375	0.645	1.234	0.364	1.376
Sorbic	0.194	0.877	0.724	1.582	0.956	2.753
			Sample 2			
Benzoic	0.0741	0.638	0.445	2.398	0.744	1.942
Sorbic	0.0697	0.559	0.421	2.179	0.601	1.894

Table 2. Recovery of the method for two samples. (I) Ketchup, (II) Chestnut sweet

Added amount	Sample (I) Recovery (%)		Sample (II) Recovery (%)	
	Benzoic acid	Sorbic acid	Benzoic acid	Sorbic acid
0.03 mM	97	98	104	101
0.06 mM	98	103	101	102
0.10 mM	103	100	99	101

Table 3. Results for the determination of benzoic acid and sorbic acid in various food products

Sample	Benzoic acid (mg/kg or mg/L)	Sorbic acid(mg/kg or mg/L)
Lemon sauce	4.36 ±0.12	235.5 ±0.9
Pickle	126.9 ±1.1	127.3 ±0.9
Fruit juice	234.9 ±0.9	178.5 ±0.7
Ketchup	463.2 ±1.0	75.3 ±1.8
Chestnut sweet	200.5 ±1.9	251.5 ±1.9
Olive paste	13.1 ±1.7	600.7 ±1.5
Pepper paste	495.5 ±1.8	10.5 ±0.4
Pepper sauce	182.8 ±1.6	188.5 ±1.2
Cake	n.d.	600.7 ±2.0

n.d.: not detected

Recovery

The accuracy of method was evaluated with two methods: (1) The peak purity was analyzed with diode array detector and (2) the known amounts of acid standards were added to food samples. Table 2 shows the recovery results for three concentration levels and for two food samples.

Application to Food Samples

The benzoic acid and sorbic acid in nine food products were quantified by using the CE method. Figures 3b and 3c illustrate the electropherograms of two food samples. In optimized conditions acids were detected within about 3.0 min. Amounts of preservatives determined in each sample were listed in Table 3. As seen from the electropherograms, there are unknown peaks in this region, but peaks of acids and internal standard are seen clearly. All food samples contain sorbic acid. In only one food sample benzoic acid was not detected. The amount of determined benzoic acid in food samples was between 4.36 and 495.5 mg/kg or mg/L. The content of sorbic acid in food samples ranged from 10.5 to 600.7 mg/kg or mg/L. The obtained data indicate that the preservatives content in foods were under permitted maximum levels.

Conclusion

In this work a simple, easy and reliable CE method was described for the simultaneous determination of benzoic and sorbic acid in various food products. The linearity of calibration curves was very good (r>0.999) and the reproducibility of method were also satisfactory (RSD < 3%). Method contain both short sample preparation time and short analysis time (~3.0 min). The matrix of samples did not interfere the analysis of analytes. In addition it contain low electrolyte and sample consumption. The silica capillaries are cheaper than chromatographic columns, easily washed between runs. Therefore the proposed CE method is used both as alternative method to HPLC for the analysis of additives in food products and routine analysis of various food for samples benzoic and sorbic acid.

References

Abedi, A.S., Mohammadi, A., Azadniya, E., Mortazavian, A.M., Khaksar, R. (2014). Simultaneous determination of sorbic and benzoic acids in milk products using an optimised microextraction technique followed by gas chromatography. *Food Additives and Contaminants Part A*, 31, 21-28

Aung, H.P., Pyell, U. (2016). In-capillary derivatization with o-phthalaldehyde in the presence of 3-mercaptopropionic acid for the simultaneous determination of monosodium glutamate, benzoic acid, and sorbic acid in food samples via capillary electrophoresis with ultraviolet detection. *Journal of Chromatography A*, 1449, 156-165

Boyce, M.C. (1999). Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography. *Journal of Chromatography A*, 847, 369-375.

Brul, S., Coote, P. (1999). Preservative agents in foods - Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology*, 50, 1-17.

Frazier, R.A., Inns, E.L., Dossi, N., Ames, J.M., Nursten, H.E. (2000). Development of a capillary electrophoresis method for the simultaneous analysis of artificial sweeteners, preservatives and colours in soft drinks. *Journal of Chromatography A*, 876, 213–220.

Han, F., He, Y.Z., Li, L., Fu, G.N., Xie, H.Y., Gan, W.E. (2008). Determination of benzoic acid and sorbic acid in food products using electrokinetic flow analysis–ion pair solid phase extraction–capillary zone electrophoresis. *Analytica Chimica Acta*, 618, 79-85.

Hsu, S.H., Hu, C.C., Chiu, T.C. (2014). Online dynamic pH junction–sweeping for the determination of benzoic and sorbic acids in food products by capillary electrophoresis. *Analytical and Bioanalytical Chemistry*, 406, 635-641.

Kuo, K.L., Hsieh, Y.Z. (1997). Determination of preservatives in food products by cyclodextrin modified capillary electrophoresis with multiwavelength detection. *Journal of Chromatography A*, 768, 334-341.

Li, B., Li, Y.K., Wang, X.Y., Wang, F., Wang, X., Wang, Y.F., Meng, X.J. (2015). Simultaneous separation and determination of organic acids in blueberry juices by capillary electrophoresis- electrospray ionization mass spectrometry. *Journal of Food Science and Technology*, 52, 5228-5235.

Lin, Y.H., Chou, S.S., Sheu, F., Shyu, Y.T. (2000). Simultaneous determination of sweeteners and preservatives in preserved fruits by micellar electrokinetic capillary

chromatography. *Journal of Chromatographic Science*, 38, 345-352.

Marsili, N., Sobrero, M.S., Goicoechea, H.C. (2003). Spectrophotometric determination of sorbic and benzoic acids in fruit juices by a net analyte signal-based method with selection of the wavelength range to avoid non-modelled interferences. *Analytical and Bioanalytical Chemistry*, 376, 126-133.

Mota, F.J.M., Ferreira, I.M.P.L.V., Cunha, S.C., Beatriz, M., Oliveira, P.P. (2003). Optimization of extraction procedures for analysis of benzoic and sorbic acids in foodstuffs. *Food Chemistry*, 82, 469-473.

Naseri, A., Farahmand, F., Sheykhizadeh S., Tabibiazar M., (2017). Coupling of air-assisted liquid–liquid microextraction method with partial least squares for simultaneous spectrophotometric determination of some preservatives. *Journal of the Iranian Chemical Society*, 14, 643-653.

Ochia, N., Sasamoto, K., Takino, M., ·Yamashita, S., Daishima, S., Heiden, A.C., Hoffmann, A. (2002). Simultaneous determination of preservatives in beverages, vinegar, aqueous sauces, and quasi-drug drinks by stir-bar sorptive extraction (SBSE) and thermal desorption GC–MS. *Analytical Bioanaytical Chemistry*, 373, 56-63

Pant, I., Trenerry, C. (1995). The determination of sorbic acid and benzoic acid in a variety of beverages and foods by micellar electrokinetic capillary chromatography. *Food Chemistry*, 53 219-226.

Petanovska-Ilievska, B., Velkoska-Markovska, L., Jankulovska, M.S. (2017). Development of reverse-phase high-performance liquid chromatography method for simultaneous determination of sodium benzoate and potassium sorbate in beverages. *Acta Chromatographica*, 29, 345-358.

Pylypiw, Jr. H.M., Grether, M.T. (2000). Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods. *Journal of Chromatography A*, 883, 299-304

Sun, B., Qi, L., Wang, M. (2014). Determination of preservatives in soft drinks by capillary electrophoresis with ionic liquids as the electrolyte additives. *Journal of Separation Science*, 37, 2248-2252.

Tfouni, S.A.V., Toledo, M.C.F. (2002). Determination of benzoic and sorbic acids in Brazilian food. *Food Control*, 13, 117-123.

Waldron, K.C., Li, J. (1996). Investigation of a pulsed-laser thermo-optical absorbance detector for the determination of food preservatives separated by capillary electrophoresis. *Journal of Chromatography B*, 683, 47-54.

Zor, Ş.D., Aşçı, B., Dönmez, Ö.A., Küçükkaraca, D.Y. (2016). Simultaneous determination of potassium sorbate, sodium benzoate, quinoline yellow and sunset yellow in lemonades and lemon sauces by HPLC using experimental design. *Journal of Chromatographic Science*, 54, 952-957.

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PHYSICOCHEMICAL, RHEOLOGICAL AND STRUCTURAL CHARACTERISTICS OF ALCOHOL PRECIPITATED FRACTION OF GUM TRAGACANTH

Abdullah Kurt

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Bitlis Eren University, Engineering-Architecture Faculty, Department of Food Engineering, 13000 Bitlis, Turkey

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Correspondence:

Abdullah KURT

E-mail: abdullahkurt48@gmail.com

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ABSTRACT

Obtaining specific polysaccharide of gum by separating other parts appears to be an approach to get a hydrocolloid with a higher quality and new functional properties. Therefore, gum tragacanth (GT) was used for these aims. The characterization of alcohol precipitated part of GT were performed in terms of physico-chemical compositions (moisture, ash, protein, color and transparency); rheological behaviors aqueous solutions at different concentration, temperature, pH, presence of salt and sucrose; and structural characterization (FTIR, XRD, DSC and SEM). Applied process has no effect on sucrose its chemical compositions which is important for preserving of progressing application. Clearer solution was obtained for purified sample which was important for sensorial properties of end-product. Rheological experiments indicated that separation insoluble part (bassorin) from GT increased viscosity and improved the stability to the different environmental conditions. FTIR experiment results confirmed that tragacanthin and bassorin is physically mixture not chemically bonded. The improvement of thermal stability of GT was also observed by DSC as a result of bassorin separation. The results suggest that extraction soluble part of GT resulted in higher rheological and structural characteristics which may help to widen its application.

Keywords: Characterization, Gum tragacanth, Purification, Rheology, Tragacanthin

Introduction

Gum tragacanth (GT) is an anionic polysaccharide produced by drying exudates from the stems and branches of Astragalus species with different compositions. GT has been accepted as GRAS at the level of 0.2–1.3% since 1961. GTgrows wildly in South West Asia particularly in Iran and Turkey (Mostafavi, Kadkhodaee, Emadzadeh, & Koocheki, 2016). GT is obtained into two different types: ribbon and flake. Ribbon type is almost opaque while the flakes are dark (Mohammadifar, Musavi, Kiumarsi, & Williams, 2006). It is also known as "kitre" in Turkey and uses in production of local ice cream and Turkish dessert, "lokma". The chemical structure is defined as a complex and highly branched polysaccharide consisting of small proportions of protein. Previous studies have reported that gum tragacanth has emulsifying and stabilizing properties in food products due to its high water binding ability and effective surface-active properties (Abdolmaleki, Mohammadifar, Mohammadi, Fadavi, & Meybodi, 2016; A. Kurt, Cengiz, & Kahyaoglu, 2016; Mostafavi et al., 2016). Pourable, creamy mouth feel and good flavour-release properties of GT were also previously stated by Balaghi, Mohammadifar, Zargaraan, Gavlighi, and Mohammadi (2011) and Levy and Schwarz (1958). The compositions and rheological properties of hydrocolloids are determinants for their usage (Balaghi, Mohammadifar, & Zargaraan, 2010).

GT consists of two main fractions: a water-soluble (tragacanthin) and an insoluble but water-swellable fraction named bassorin (Balaghi et al., 2011). The ratio of these parts strongly depends on variety which resulted in different flow, structural characteristics and also different functionalities and applications for food systems. In order to acquire GT with a higher quality and independently from species, we aimed to remove insoluble part bassorin from the structure. It is expected that removing bassorin will provide enhancement of interaction between polymer chains and more stable solution against different environmental conditions may be obtained. In addition to stable solutions property, clearer solution is also desired in terms of visual acceptability. Balaghi et al. (2010) also defined the best quality gum tragacanth as tasteless, whitish in color, and translucent in appearance, giving an aqueous solution of high-viscosity, free from sand or bark. Therefore, in the last years, scientific researches have focused on the quality improvement of hydrocolloids by separating the constituents from the main part of gum such as insoluble materials and impurities (Razmkhah, Mohammadifar, Razavi, & Ale, 2016). The widely applied method consists of following main steps: mixing hydrocolloids in a water with a specific ratio, removing insoluble matter by centrifugation and obtaining specific polysaccharide from the supernatant with different ways such as freeze or spray drying, precipitation with alcohol or separate with the form of complexes by using copper or barium (Abdullah Kurt & Kahyaoglu, 2017b, 2017c; Razmkhah et al., 2016). Bassorin separated GT had previously obtained from supernatant with different drying methods: spray drying (Koshani, Aminlari, Niakosari, Farahnaky, & Mesbahi, 2015) and freeze drying (Gavlighi, Meyer, Zaidel, Mohammadifar, & Mikkelsen, 2013; Mohammadifar et al., 2006). The alcohol precipitation methods were widely used for extraction due to the relatively cost effective, healthier and quick acquiring properties as compared with other mention methods. To the best of our knowledge there are no reports about alcohol precipitation of tragacanthin and their detailed characterization of product.

The easy separation of tragacanthin and bassorin indicated that two polysaccharides are in a physical mixture and not chemically bonded (Balaghi et al., 2010; Koshani et al., 2015). Therefore, to isolate the specific polysaccharide tragacanthin was aimed by applying ethanol precipitation method as a simple and feasible approach in this study. Increased quality will also provide to get higher viscosity at relative low concentrations which are desired characteristics for hydrocolloids. Physicochemical, rheological properties in different pH, salt and sugar concentration and structural variations were evaluated to observe the effect of applied process on potential application of GT.

Materials and Methods

Gum tragacanth (GT) was kindly donated by Incom (Mersin, Turkey). All chemicals used in this study were of analytical reagent grade. The ethanol, acetone, sodium chloride, calcium chloride, sucrose, lactose were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The pH buffer solutions were obtained from Merck (Darmstadt, Germany).

Removing of Bassorin from Gum Tragacanth

Ten grams of crude gum tragacanth was stirred in 1 L of distilled water for 1 h at room temperature, followed by centrifugation (5000 rpm, 10 min) to remove basssorin. The supernatants were mixed with absolute ethanol at a ratio of 1:1 (v/v) to precipitate of tragacanthin. The resultant pellets were washed successively with absolute ethanol and acetone and then subsequently dried with a forced air dryer (Mikrotest, Turkey) at 45°C overnight. The dried samples were milled, sieved, stored in an airtight bottle at room temperature and coded as BSGT (Bassorin- separated gum tragacanth).

Physicochemical Analyses

Solubility analyses were performed to determine ratio of soluble to insoluble fraction of crude gum tracagacanth (GT) using the method of Li et al. (2014). 0.1 g GT was dispersed in 24.90 g distilled water and the mixture was agitated at room temperature for 1 h. Then mixture was centrifuged for 20 min at 4500 rpm, and 10.00 g of supernatant was dried to a constant weight at 105°C. The solubility was calculated as follows:

$$Solubility\ (\%) = \frac{m \times 2.5}{w} \times 100\% \quad \textbf{1}$$

where m is the dry matter content of dried supernatant and w is the total mass of the sample.

The moisture and ash content of samples were analyzed by using gravimetric methods at 105 and 500°C, respectively. Kjeldahl method was performed for protein determination by the applying the nitrogen conversion factor of 6.25 (Balaghi et al., 2010). Fat content was analyzed by soxhlet method. pH values of gum solutions (1%) were measured with pH meter (Eutech Instruments, pH 700, Singapore) at room temperature. L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values of powders were determined by a Minolta Chromameter (CR-400, Minolta Camera Co., Osaka, Japan) to calculate the whiteness index (WI) of the powders as follows (Abdullah Kurt & Kahyaoglu, 2017a):

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad \textbf{2}$$

The transparency of 1% gum solution was evaluated by percent transmittance at 500 nm against distilled water blank with a Cary 60 UV–visible spectrophotometer (Agilent Technologies, Victoria, Australia) (Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002).

Rheological Properties

Analyses of the rheological properties of gum solutions were performed using rheometer (HAAKE Mars III; Thermo Scientific, Germany) equipped with a Peltier heating system in a cone and plate configuration (diameter: 35 mm, cone angle: 2°, gap size: 0.105 mm).

Different concentrations of the samples (1.0 and 2.0%) were prepared in distilled water with stirring for 1 h using a magnetic stirrer at room temperature. For each test, samples were allowed to equilibrate for 1 min at the desired temperature (5, 10, 25 and 45°C). Samples were sheared continuously at a rate ranging from 0 to 300 s^{-1} in 3 min for fitting the data to the Ostwald-de Waele model as follows:

$$\tau = K.\dot{\gamma}^n \quad \textbf{3}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$), and n is the flow behavior index (dimensionless).

The flow curves were obtained by registering shear stress at shear rates from 0 to 300 s−1 (forward) in 180 s and down in 180 s from 300 to 0 s−1 (backward). Thixotropic areas, A_t were obtained using data acquisitions software. A_t values were calculated using the equation as follows:

$$A_t = \frac{(A_{up} - A_{down})}{A_{up}} x100 \quad \textbf{4}$$

Solutions (1%) were also prepared with different environments: pH (4.0 and 5.5), salt (NaCl and $CaCI_2$; 10, 50 and 100 mM) and sugar (sucrose and lactose; 2, 4 and 6%). Steady flow characteristics of these solutions were determined at 25 °C (0 to 300 s^{-1} in 3 min).

Structural Characterization of GT and BSGT

Fourier-transform infrared (FTIR) spectroscopy analyses

The FT-IR spectra of samples were recorded on a spectrophotometer (Perkin Elmer, Model Spectrum Two, Ohio, USA) that was fitted with a Miracle Single-Reflection Diamond ATR device in the wavelength range of 4000–650 cm^{-1} with a spectral resolution of 4 cm^{-1}.

X ray diffraction (XRD) analyses

The XRD patterns of the samples were obtained using a Rigaku SmartLab X-ray diffractometer (40 kV, 30 mA) with a scanning rate of 2°/min from 5° to 45° (2θ range).

Differential scanning calorimetry (DSC) analyses

The thermal analyses of the samples were performed with a model DSC 4000 (Perkin Elmer, USA). Five milligrams of the sample in an aluminium pan with an empty reference pan were scanned in a nitrogen atmosphere (20 mL/min) at a heating rate of 10°C/min in a temperature range of 30–350°C.

Scanning electron microscopy (SEM) observation

The SEM photomicrographs were recorded using a scanning electron microscope (JSM-7001F, JEOL, Japan). The samples were coated with gold-palladium (Quorum SC7620, England) before observation under the microscope.

Results and Discussion

Physicochemical Analyses

The soluble/insoluble ratio is important parameter for the quality of GT which was determined as 2.6 in this study, consistent with the reported study by Gavlighi et al. (2013). Removing bassorin fraction of GT increased this ratio to the highest degree and the flow properties of GT changed significantly. This ratio was reported between 0.5-3.15 for different species of GM in previous studies (Balaghi et al., 2010). At the end of this purification studies, 35% of soluble part of GT was obtained by alcohol precipitation method. Ethanol soluble minor fraction, arabinogalactan could be attributed to the decrease in yield which lost with ethanol instead of precipitation. The extraction yield of hydrocolloids varies depending upon the origin and extraction method. Alcohol extraction method of GT is the first attempt and the yield obtained in this study is comparable with the other purified hydrocolloids such as glucomannan and galactomannan (Abdullah Kurt & Kahyaoglu, 2017b). Spray (Koshani et al., 2015) and freeze (Mohammadifar et al., 2006) drying methods were also conducted to the supernatant phase of GT solutions but there were no information about yield of these methods.

Table 1 summarizes some of the physicochemical properties of GT and BSGT. Total solid, ash and protein contents of GT were not affected from the purification process and obtained results were consistent with the reported study for GT (Mohammadifar et al., 2006) . In similar environmental conditions, different moisture content of hydrocolloid is an evidence of different water polysaccharide interaction and different storage stability (Balaghi et al., 2010). Ash content indicator of mineral content of gum shows variations related to the composition of the soil in which GT grow. During the precipitation of polymeric structure by alcohol, ash constituents were also gained. The presence of protein which plays a pivotal role in gum surface activity makes gum a candidate for emulsification and surface tension reduction. The lower protein content may indicate the purity of hydrocolloids. However purification process did not reduce protein content of GT which could be attribute to the linkages of protein to the molecular structure of hydrocolloids such as gum Arabic (Amid & Mirhosseini, 2012; Brummer, Cui, & Wang, 2003; Seyed Mohammad Ali Razavi, Cui, & Ding, 2016). Small amounts of protein which bounded to the polysaccharide was stated by by Farzi et al. (2011). The result is important for the preservation of this functional property of GT with purification process because previous studies have shown that GT has emulsifying and stabilizing properties, which means that GT could be evaluated for obtaining stable oil-in-water emulsions (Abdolmaleki et al., 2016). The samples were not included fat content. Aqueous suspension of samples gave an acidic pH which found as 5.40 and 5.82 for GT and BSGT, respectively. Acidic nature of gum indicated the presence of uronic acid in polysaccharide structure (Nep & Conway, 2011). As expected, removing insoluble part had no effect on pH. pH of GT which depends on origins, agricultural history and constituents was reported between 5.2-5.4 by Teimouri, Abbasi, and Sheikh (2016). Cleared and more stable solutions were desired for gum solutions (Abdullah Kurt & Kahyaoglu, 2017b). The whiteness index (WI) of powder and transparency of solutions values of GT were presented in Table 1. These parameters of GT increased after purification as a result of removing bassorin and alcohol treatment. The presence of insoluble fragment of GT was responsible for turbidity. Whitish in color and translucent in appearance properties which were the quality parameters of GT were improved by purification study. According to findings of physicochemical analyses it could be said that purification treatment to GT had no effect on chemical constituents but increased the solution properties of GT.

Table 1. Physicochemical properties of crude (GT) and bassorin separated (BSGT) gum tragacanth

Properties	GT	BSGT
Total Solids (g/100g)	89.53±0.67	90.01±0.01
Ash (g/100g)	3.41±0.02	3.30±0.03
Protein (g/100g)	1.67±0.03	1.72±0.12
pH	5.40±0.00	5.82±0.02
Whiteness ındex (WI)	81.05±0.23	90.51±0.26
Transparency (%)	8.57±0.00	47.72±0.00

(n=3) ± SD

Rheological Properties

Steady-shear flow behavior of gum tragacanth

The variation in apparent viscosity and Ostwald de Waele model parameters (*K*, *n*) of gum tragacanth with the removing bassorin fraction was shown in Table 2 for the different concentration. The viscosity increment with purification was observed for GT. Viscosity is the one of the main parameter to evaluate the quality of polymers (Abdullah Kurt & Kahyaoglu, 2015). Intermolecular forces and polymer interactions were responsible for the solution viscosity (A. Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009). Therefore, the interaction between polymer chains and quality of GT increased as a result of removing insoluble part, bassorin.

Table 2. Apparent viscosity (shear rate: $30s^{-1}$), Ostwald de Waele model parameters (*K*, *n*), thixotropic area (A_t), activation energy (E_a) of samples.

Sample	Concentration	Apparent Viscosity (Pas)	*K* (Pas)	*n*	A_t (Pa/s)	E_a (kJ/mol)
GT	**1%**	0.17±0.00	0.94±0.04	0.51±0.01	16.16±0.21	20.20±0.26
BSGT		0.25±0.00	1.49±0.02	0.48±0.00	14.46±0.62	23.83±0.23
GT	**2%**	1.02±0.00	9.82±0.38	0.33±0.00	394.55±0.63	10.22±0.31
BSGT		1.36±0.02	14.25±0.12	0.30±0.00	236.15±0.49	14.18±0.25

(n=3) ± SD

The data was fitted by the Ostwald-de Waele model successfully (R2 ≥ 0.99). Consistency coefficient (*K*) is associated with the higher viscosity of the solution since *K* values indicative of the viscous character of the polymeric system. The larger *K* values of BSGT were another result of better interaction among the molecules of the polysaccharide. *n* values were ranging from 0.30 to 0.51, namely, lower than unity was an evidence of pseudoplasticity. Shear-thinning behavior of GT was previously reported by Farzi et al. (2015). This fact infers that as shear rate increases, the randomly positioned chains of polymer molecules become aligned in the direction of flow, resulting in less interaction among adjacent polymer chains (Arash Koocheki, Taherian, & Bostan, 2013). Decreased in *n* value by purification indicated improved pseudoplastic characteristics of GT. In addition, increasing gum concentration resulted in increase of *K* values whereas *n* values decreased. Higher content of total solids in solution causes an increase in consistency due to the decrement of intermolecular motion as a result of hydrodynamic forces (Capitani et al., 2015). Similar flow properties of GT was reported previously by Chenlo, Moreira, and Silva (2010).

The differences between upward and downward curves which are the measure of the extent of thixotropy indicate time-dependent rheological characteristics of sample (Seyed M. A. Razavi & Karazhiyan, 2009). Table 2 shows the calculated hysteresis loop area for GT solutions. Magnitude of the areas decreased slightly at 1% and markedly at 2% by removing bassorin which mean that BSGT structure was less damaged from the shear increase and also recovered to the first structure closer than GT (A. Kurt et al., 2016; Roopa & Bhattacharya, 2009).

The temperature dependency of the consistency index was evaluated by using the Arrhenius model and activation energy (E_a) of purified salep samples were exhibited in Table 2. E_a is related to chain flexibility and is an indicator of ability of molecule movement as temperature increases (A. Koocheki et al., 2009; Arash Koocheki et al., 2013). The higher sensitivity of viscosity to temperature changes implies higher E_a value. At all concentrations, E_a values increased with the removing bassorin fraction mean that BSGT flow more easily when the temperature is increased due to the higher molecular movement than GT. It could be stated that insoluble fraction (bassorin) of GT had a favorable effect on temperature stability for viscosity. Increasing concentration decreased E_a values of GT and BSGT which related to decrease in chain flexibility as a result of decreasing the space for a molecule to flow (A. Koocheki et al., 2009). Similar with reported study by (Mohammadifar et al., 2006) GT exhibited lower E_a values than tragacanthin which was the soluble part of GT obtained by freeze drying. The results above suggest that, in order to keep stable viscosity, temperature control is critical for BSGT in food applications.

Effect of pH, Salt and Sugar on Rheological Properties

pH, salt and sugar effect on consistency coefficient of GT and BSGT shown in Figure1 were evaluated because gums are usually processed under different environmental conditions. As shown in Figure 1A, pH effects were investigated to gain insight into the influence removing insoluble part of GT on the polyelectrolyte nature and solution rheology. Decreasing pH values decreased *K* values of GT but increased consistency coefficient of BSGT. The variation of viscosity related to the pH is attributed to the change in ionization of the groups in molecule such as carboxyl groups and conformational changes in the molecule (Abdullah Kurt & Kahyaoglu, 2017b). Sharp increment of a gum was attributed to the ionization of its carboxyl groups (Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000; Wu, Ding, Jia, & He, 2015). The decrease in viscosity at lower pH values of GT could be a result of insoluble part bassorin that effect the polymer conformation because BSGT exhibited increasing trend when pH decreased.

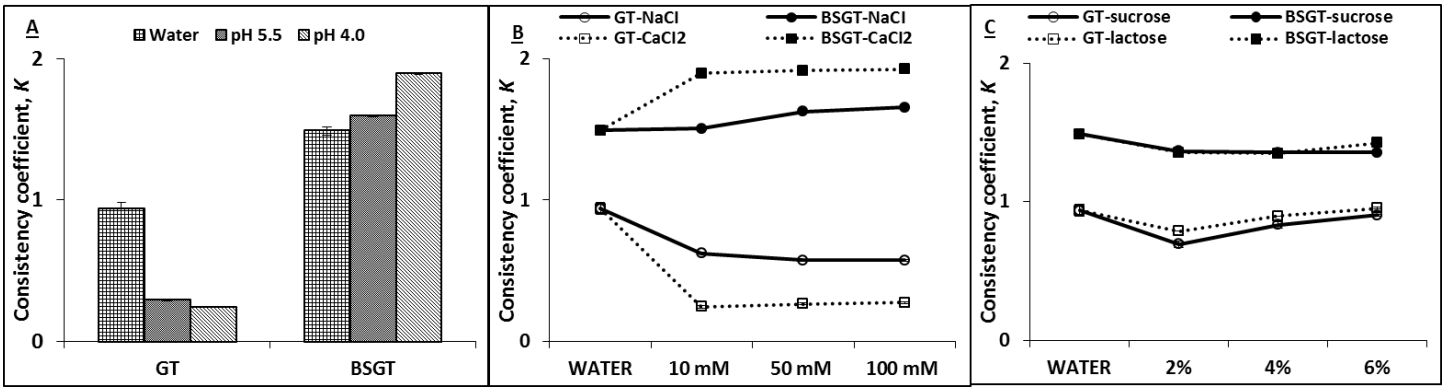

Figure 1. pH (A), salt-NaCI, $CaCI_2$ (B), sugar-sucrose, lactose (C) effect on consistency coefficent of GT (gum tragacanth) and BSGT (bassorin separated gum tragacanth)

The ionic strength of the solution is an important parameter which has an effect on the networking mechanism of charged biopolymers (Balaghi et al., 2011). The changing consistency of studied gum in different salt and their concentration indicated the polyelectrolyte properties in solution of GT and BSGT (Abdullah Kurt & Kahyaoglu, 2017a; Medina-Torres et al., 2000). As shown in Figure 1B, both salt types (NaCI and $CaCI_2$) addition decreased the consistency coefficient of GT similar with the reported studies (Balaghi et al., 2011; Mohammadifar et al., 2006) but the salts led to an increase in this property of BSGT which exhibited rapid reduction and increment, respectively for 10mM salt concentration. At increasing concentration, viscosity values of both gum exhibited stable trends. Comparatively, the viscosity variation is more sensitive to $CaCI_2$ than NaCI which consistent with the reported study for tara gum due to the stronger effect of divalent salt $CaCI_2$ than NaCI (Capitani et al., 2015). Gum tragacanth is known as a branched, heterogeneous, and anionic carbohydrate (Balaghi et al., 2010). Negatively charged groups causes expansions of molecules in solution which lead to the higher viscosity if the solutions are not included any salts. However addition positive ions are resulted in different flow behavior. These observations were attributed to the forming junctions and reinforcing interaction between the molecules with the aid of ions for BSGT which resulted viscosity increment (Farahnaky, Shanesazzadeh, Mesbahi, & Majzoobi, 2013). On the other hand, insoluble part could be exhibited hurdle effect between the molecules which lost its expansion as a result of added positive ions, resulted viscosity decrement.

Addition of 2% sucrose and lactose resulted sharply decrement in *K* values of both gums (Figure1C). Increase in concentration provided an increase in viscosities which resemble its water condition for GT. However increasing sugar concentration after 2% did not change solution viscosity of BSGT. Extending of polymer molecules was reduced by sugar due to the decrement of available water for gum hydration which leads to the viscosity drop (Wu et al., 2015). Another explanation is that decrease in the size of polymers as a result of the decrease in the macromolecular association at the presence of sugar, which was observed for locust bean gum and purified salep glucomannan (Abdullah Kurt & Kahyaoglu, 2017a; Richardson, Willmer, & Foster, 1998). It can be concluded that the viscosity of BSGT in different conditions showed higher values than GT indicated that removing insoluble part fraction of GT improved gum quality (i), provided stability (ii) and decreased gum requirement to obtained desired viscosities (iii).

Structural Characterization of GT and BSGT

FTIR spectroscopy analysis

The FTIR spectrum of GT and BSGT was shown in Fig. 2A to gain further insights effect of removing bassorin on the structure and bonding in the polymer. Broad band around 3330 cm^{-1} was due to stretching vibrations of –OH group related to free, inter, and intra-molecular bound hydroxyl groups and the band at 2940 cm^{-1} is an indicator of C–H stretching (Antoniou, Liu, Majeed, & Zhong, 2015; Abdullah Kurt & Kahyaoglu, 2015). The peaks at 1744 cm^{-1} could be assigned to carbonyl stretching vibrations (C=O stretching of −COOH) (Zarekhalili, Bahrami, Ranjbar-Mohammadi, & Milan, 2017).The intensity of the band in the region 1623–1644 cm^{-1} attributed to the presence of protein in the structure (Chua et al., 2012). The observed different rheological behavior of GT and BSGT at different salt and pH environment was the evidence of the presence of uronic acid in structure which related to wavenumber about 1400 cm^{-1} (Abdullah Kurt & Kahyaoglu, 2017c; Seyed Mohammad Ali Razavi, Cui, Guo, & Ding, 2014). The bands at 1245,

1079 and 1024 cm^{-1} were related to stretching vibrations of C-O in polyols, ethers and alcohol groups, respectively (Zarekhalili et al., 2017). The peaks between 800 and 1200 cm^{-1} were pointed out the finger print region for carbohydrates (Nep & Conway, 2011). FTIR spectra of bassorin separated gum tragacanth did not show significant difference in terms of intensity, the position of absorption bands, no shift of peaks and new peak occurrence in comparison to GT, indicating applied process to the GT to remove bassorin preserved the chemical structure of gum. Therefore, FTIR experiment results confirmed that two different fractions of GT, tragacanthin and bassorin are mixture in physical form instead of chemical (Farzi et al., 2015).

XRD analyses

XRD experiments were conducted to determine crystallinity of GT and effect on purification on that (Figure 2B). XRD pattern of samples showed a broad peak (at 2θ about 20°) due to amorphous nature of GT which consistent with the reported studies (Singh & Sharma, 2017; Singh, Varshney, Francis, & Rajneesh, 2016). Bassorin separation from GT resulted in lower broad peak intensity with an amorphous nature. Amorphous materials exhibit more hygroscopic characteristics than crystalline structure due to the absorption properties which caused to increase of exposed absorption sites (Abdullah Kurt & Kahyaoglu, 2017a; Xiao et al., 2015). Therefore GT and BSGT are soluble in water even at room temperature.

DSC analyses

DSC thermograms of GT and BSGT were shown in Figure 2C. There were two main peaks. The first transition endothermic peak could be ascribed to be due to vaporization of moisture or bound water present in the samples (Abdullah Kurt & Kahyaoglu, 2017c). The lack of melting endothermic peak also confirmed the amorphous nature of samples. The second peak exhibited exothermic transition which was the main decomposition of molecular chain (Li et al., 2014). Applied process made this peak narrower which indicated relative purity of BSGT (Pawar & Lalitha, 2014). The onset of the exothermic peak temperature (T_{onset}) and the maximum temperature (T_{max}) of GT increased from 237.72 to 251.98 °C and from 258.68 to 263.43 °C, respectively, as a result of bassorin separation, which showed the improvement of thermal stability due to the enhancement of hydrogen bonding interaction in the structure of BSGT. The decomposition of molecular chain reported between 250 and 280 °C for GT (Zarekhalili et al., 2017) which agree with our results. The higher decomposition temperature of BSGT can be correlated with its stable structure of BSGT during various food processes that involves higher temperatures.

SEM analyses

SEM micrographs of samples were presented in Figure 4D at 500× magnification. The shape of granule and surface morphology variations as a consequence of ethanol treatment to GT were observed with this study. Firstly, shape of granule exhibited good agreement with reported study for GT (Alijani, Balaghi, & Mohammadifar, 2011). The images indicated that applied process preserved shape of granules and no destructive effect was observed as expected. The result was another confirmation of coexisting bassorin and tragacanthin in physically. The smoother surface of granules appeared with ethanol treatment which is consistent with the study (Abdullah Kurt & Kahyaoglu, 2015) and responsible for the higher whiteness of BSGT.

Conclusions

The bassorin separation from gum tragacanth had no effect on its chemical structure but solution transparency was improved. The viscosities of bassorin separated sample solution are higher than those of gum tragacanth solutions at the same gum concentration, pH, salt and sugar conditions. The more stable gum solution was obtained by purification at lower pH and higher salt concentrations. Thermal stability of gum tragacanth was also improved with this process. The results obtained in this study could be useful for widening the application of gum tragacanth in the food, chemical and polymer industries due to the better rheological and structural characteristics.

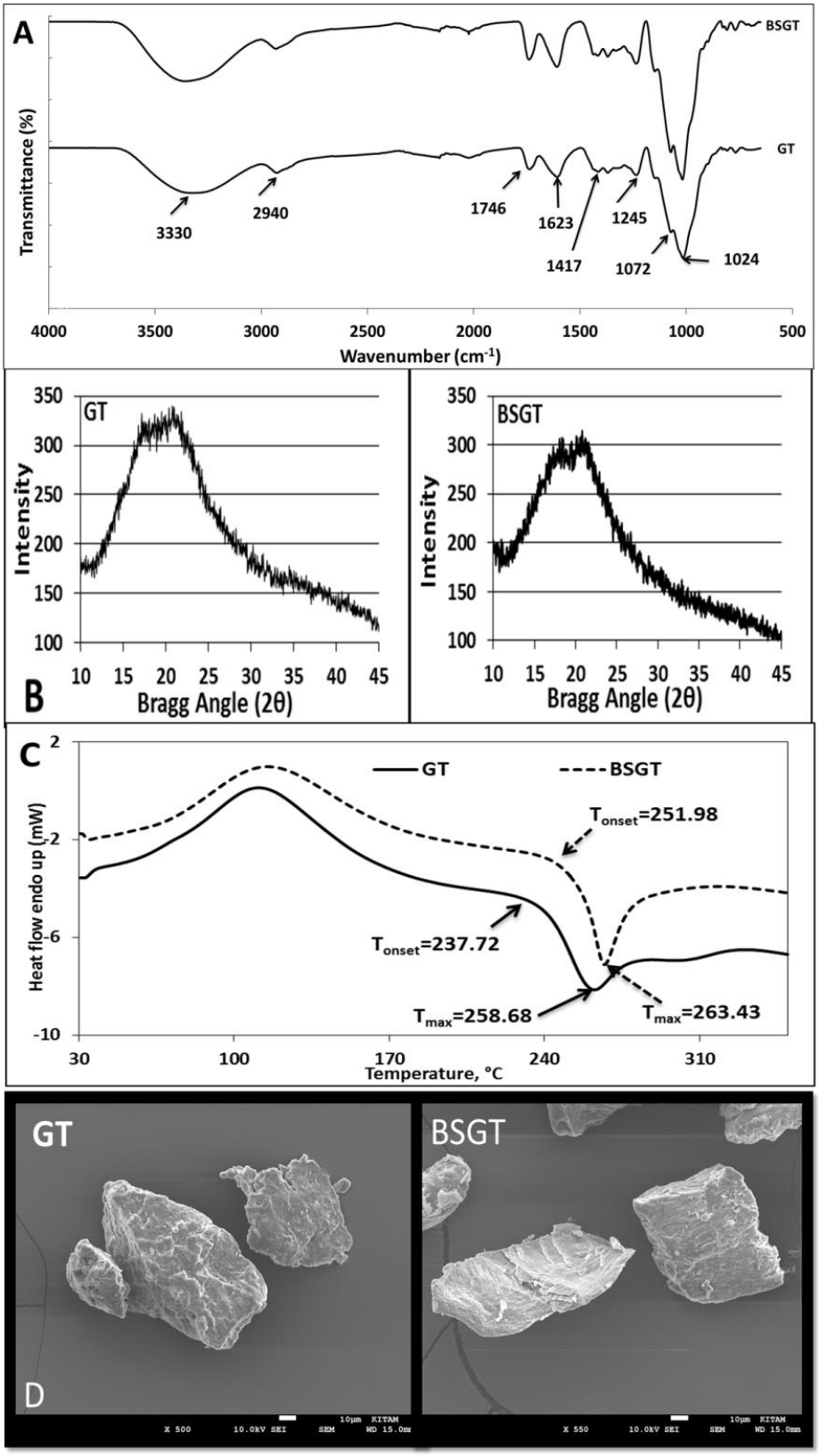

Figure 2. FTIR spectra (A), XRD patterns (B), DSC thermograms (C) and SEM images (D) of GT (gum tragacanth) and BSGT (bassorin separated gum tragacanth)

References

Abdolmaleki, K., Mohammadifar, M.A., Mohammadi, R., Fadavi, G., Meybodi, N.M. (2016). The effect of pH and salt on the stability and physicochemical properties of oil-in-water emulsions prepared with gum tragacanth. *Carbohydrate Polymers*, 140, 342-348.

Alijani, S., Balaghi, S., Mohammadifar, M.A. (2011). Effect of gamma irradiation on rheological properties of polysaccharides exuded by A. fluccosus and A. gossypinus. *International Journal of Biological Macromolecules*, 49(4), 471-479.

Amid, B.T., Mirhosseini, H. (2012). Emulsifying Activity, Particle Uniformity and Rheological Properties of a Natural Polysaccharide-Protein Biopolymer from Durian Seed. *Food Biophysics*, 7(4), 317-328.

Antoniou, J., Liu, F., Majeed, H., Zhong, F. (2015). Characterization of tara gum edible films incorporated with bulk chitosan and chitosan nanoparticles: A comparative study. *Food Hydrocolloids*, 44, 309-319.

Balaghi, S., Mohammadifar, M.A., Zargaraan, A. (2010). Physicochemical and Rheological Characterization of Gum Tragacanth Exudates from Six Species of Iranian Astragalus. *Food Biophysics*, 5(1), 59-71.

Balaghi, S., Mohammadifar, M.A., Zargaraan, A., Gavlighi, H.A., Mohammadi, M. (2011). Compositional analysis and rheological characterization of gum tragacanth exudates from six species of Iranian Astragalus. *Food Hydrocolloids*, 25(7), 1775-1784.

Brummer, Y., Cui, W., Wang, Q. (2003). Extraction, purification and physicochemical characterization of fenugreek gum. *Food Hydrocolloids, 17*(3), 229-236.

Capitani, M.I., Corzo-Rios, L.J., Chel-Guerrero, L.A., Betancur-Ancona, D.A., Nolasco, S.M., Tomás, M.C. (2015). Rheological properties of aqueous dispersions of chia (Salvia hispanica L.) mucilage. *Journal of Food Engineering*, 149, 70-77.

Chenlo, F., Moreira, R., Silva, C. (2010). Rheological properties of aqueous dispersions of tragacanth and guar gums at different concentrations. *Journal of Texture Studies*, 41(3), 396-415.

Chua, M., Chan, K., Hocking, T.J., Williams, P.A., Perry, C.J., Baldwin, T. C. (2012). Methodologies for the extraction and analysis of konjac glucomannan from corms of Amorphophallus konjac K. Koch. *Carbohydrate Polymers*, 87(3), 2202-2210.

Farahnaky, A., Shanesazzadeh, E., Mesbahi, G., Majzoobi, M. (2013). Effect of various salts and pH condition on rheological properties of Salvia macrosiphon hydrocolloid solutions. *Journal of Food Engineering*, 116(4), 782-788.

Farzi, M., Saffari, M.M., Emam-Djomeh, Z., Mohammadifar, M.A. (2011). Effect of ultrasonic treatment on the rheological properties and particle size of gum tragacanth dispersions from different species. *International Journal of Food Science and Technology*, 46(4), 849-854.

Farzi, M., Yarmand, M.S., Safari, M., Emam-Djomeh, Z., Mohammadifar, M.A. (2015). Gum tragacanth dispersions: Particle size and rheological properties affected by high-shear homogenization. *International Journal of Biological Macromolecules*, 79, 433-439.

Gavlighi, H.A., Meyer, A.S., Zaidel, D.N.A., Mohammadifar, M.A., Mikkelsen, J.D. (2013). Stabilization of emulsions by gum tragacanth (Astragalus spp.) correlates to the galacturonic acid content and methoxylation degree of the gum. *Food Hydrocolloids*, 31(1), 5-14.

Kobayashi, S., Tsujihata, S., Hibi, N., Tsukamoto, Y. (2002). Preparation and rheological characterization of carboxymethyl konjac glucomannan. *Food Hydrocolloids, 16*(4), 289-294.

Koocheki, A., Mortazavi, S.A., Shahidi, F., Razavi, S.M.A., Taherian, A.R. (2009). Rheological properties of mucilage extracted from Alyssum homolocarpum seed as a new source of thickening agent. *Journal of Food Engineering*, 91(3), 490-496.

Koocheki, A., Taherian, A.R., Bostan, A. (2013). Studies on the steady shear flow behavior and functional properties of Lepidium perfoliatum seed gum. *Food Research International, 50*(1), 446-456.

Koshani, R., Aminlari, M., Niakosari, M., Farahnaky, A., Mesbahi, G. (2015). Production and properties of tragacanthin-conjugated lysozyme as a new multifunctional biopolymer. *Food Hydrocolloids*, 47, 69-78.

Kurt, A., Cengiz, A., Kahyaoglu, T. (2016). The effect of gum tragacanth on the rheological properties of salep based ice cream mix. *Carbohydrate Polymers*, *143*, 116-123.

Kurt, A., Kahyaoglu, T. (2015). Rheological properties and structural characterization of salep improved by ethanol treatment. *Carbohydrate Polymers*, 133, 654-661.

Kurt, A., Kahyaoglu, T. (2017a). Gelation and structural characteristics of deacetylated salep glucomannan. *Food Hydrocolloids*, 69, 255-263.

Kurt, A., Kahyaoglu, T. (2017b). Purification of glucomannan from salep: Part 1. Detailed rheological characteristics. *Carbohydrate Polymers*, 168, 138-146.

Kurt, A., Kahyaoglu, T. (2017c). Purification of glucomannan from salep: Part 2. Structural characterization. *Carbohydrate Polymers*, 169, 406-416.

Levy, G., Schwarz, T. W. (1958). Tragacanth Solutions I. *Journal of the American Pharmaceutical Association (Scientific ed.)*, 47(6), 451-454.

Li, J., Ye, T., Wu, X., Chen, J., Wang, S., Lin, L., Li, B. (2014). Preparation and characterization of heterogeneous deacetylated konjac glucomannan. *Food Hydrocolloids*, 40, 9-15.

Medina-Torres, L., Brito-De La Fuente, E., Torrestiana-Sanchez, B., Katthain, R. (2000). Rheological properties of the mucilage gum (*Opuntia ficus indica*). *Food Hydrocolloids*, 14(5), 417-424.

Mohammadifar, M.A., Musavi, S.M., Kiumarsi, A., Williams, P.A. (2006). Solution properties of targacanthin (water-soluble part of gum tragacanth exudate from Astragalus gossypinus). *International Journal of Biological Macromolecules*, 38(1), 31-39.

Mostafavi, F.S., Kadkhodaee, R., Emadzadeh, B., Koocheki, A. (2016). Preparation and characterization of tragacanth-locust bean gum edible blend films. *Carbohydrate Polymers*, 139, 20-27.

Nep, E.I., Conway, B.R. (2011). Physicochemical characterization of grewia polysaccharide gum: Effect of drying method. *Carbohydrate Polymers*, 84(1), 446-453.

Pawar, H.A., Lalitha, K.G. (2014). Isolation, purification and characterization of galactomannans as an excipient from Senna tora seeds. *International Journal of Biological Macromolecules*, 65, 167-175.

Razavi, S.M.A., Cui, S.W., Ding, H. (2016). Structural and physicochemical characteristics of a novel water-soluble gum from Lallemantia royleana seed. *International Journal of Biological Macromolecules*, 83, 142-151.

Razavi, S.M.A., Cui, S.W., Guo, Q., Ding, H. (2014). Some physicochemical properties of sage (Salvia macrosiphon) seed gum. *Food Hydrocolloids*, 35, 453-462.

Razavi, S.M.A., Karazhiyan, H. (2009). Flow properties and thixotropy of selected hydrocolloids: Experimental and modeling studies. *Food Hydrocolloids*, 23(3), 908-912.

Razmkhah, S., Mohammadifar, M.A., Razavi, S.M.A., Ale, M.T. (2016). Purification of cress seed (Lepidium sativum) gum: Physicochemical characterization and functional properties. *Carbohydrate Polymers*, 141, 166-174.

Richardson, P.H., Willmer, J., Foster, T.J. (1998). Dilute solution properties of guar and locust bean gum in sucrose solutions. *Food Hydrocolloids*, 12(3), 339-348.

Roopa, B.S., Bhattacharya, S. (2009). Characterisation and modelling of time-independent and time-dependent flow behaviour of sodium alginate dispersions. *International Journal of Food Science and Technology*, 44(12), 2583-2589.

Singh, B., Sharma, V. (2017). Crosslinking of poly(vinylpyrrolidone)/acrylic acid with tragacanth gum for hydrogels formation for use in drug delivery applications. *Carbohydrate Polymers*, 157, 185-195.

Singh, B., Varshney, L., Francis, S., Rajneesh. (2016). Designing tragacanth gum based sterile hydrogel by radiation method for use in drug delivery and wound dressing applications. *International Journal of Biological Macromolecules*, 88, 586-602.

Teimouri, S., Abbasi, S., Sheikh, N. (2016). Effects of gamma irradiation on some physicochemical and rheological properties of Persian gum and gum tragacanth. *Food Hydrocolloids*, 59, 9-16.

Wu, Y., Ding, W., Jia, L., He, Q. (2015). The rheological properties of tara gum (Caesalpinia spinosa). *Food Chemistry*, 168, 366-371.

Xiao, M., Dai, S., Wang, L., Ni, X., Yan, W., Fang, Y., Corke H., Jiang F. (2015). Carboxymethyl modification of konjac glucomannan affects water binding properties. *Carbohydrate Polymers*, 130, 1-8.

Zarekhalili, Z., Bahrami, S.H., Ranjbar-Mohammadi, M., Milan, P.B. (2017). Fabrication and characterization of PVA/Gum tragacanth/PCL hybrid nanofibrous scaffolds for skin substitutes. *International Journal of Biological Macromolecules Part A*, 94, 679-690.

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PRELIMINARY STUDY OF DNA EXTRACTION FROM BULGARIAN HONEYS AND ITS AMPLIFICATION BY PCR FOR BOTANICAL IDENTIFICATION

Ralitsa Balkanska[1], Katerina Stefanova[2], Radostina Stoikova – Grigorova, Vanya Manolova[3]

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[1] Institute of Animal Science, Kostinbrod, 2232 Kostinbrod, Bulgaria

[2] Agrobioinstitute, 1164 Sofia, Bulgaria

[3] Central Laboratory of Veterinary Control and Ecology, 1528 Sofia, Bulgarian Food Safety Agency, Bulgaria

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Correspondence:

Ralitsa BALKANSKA

E-mail: r.balkanska@gmail.com

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ABSTRACT

The physiochemical and biological properties of honey are directly associated to its floral origin. Some current commonly used methods for identification of botanical origin of honey involve melissopalynological analysis or other analytical methods. However, these methods can be less sensitive and time-consuming. DNA-based methods have become popular due to their quickness and reliability. In this respect, the purpose on the present study is to compare two DNA extraction kits – DNeasy Plant Mini Kit (Qiagen) and GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) and CTAB method combined with different pre-treatment procedures and applied to 3 multifloral and 2 monofloral honey samples. The DNA extraction and amplification was carried out for all honey samples with similar results in each replication. The extracted DNA was amplified by PCR using plant specific primers rbcL gene and trnH-psbA spacer. The result obtained confirming that the DNA extracted has good quality for analysis of PCR products and that it can be used for botanical identification of honey.

Keywords: Honey, rbcL, trnH-psbA spacer, DNA extraction, Botanical identification

Introduction

There are a number of methods available for the determination of botanical origin of the honey. The traditional method for honey botanical determination (melissopalynology) is based on microscopic analysis of honey pollen composition. However, this method is time-consuming, requires experts in the botany with adequate skills and experience to identify pollen grains based on its different morphologies. In the recent years, in the process of development are other research approaches for determination of honey botanical origin such as volatile compounds (Guyot et al., 1999; Radovic et al., 2001; Soria et al., 2004). Furthermore, combination of some physicochemical parameters, elemental composition and protein content may be used for honey identification (Liberato et al., 2013). Interesting research task is determination of mineral and trace elements in honey for determination of botanical origin (Fernandez-Torres et al., 2005; Hernandez et al., 2005; Latorre et al., 1999). The markers for identification of different honey types are flavonoids, aromatic compounds and sugars (Wang and Li, 2011). In recent years, modern DNA methods for determination of honey botanical origin are needed. DNA barcode is a technology enables the identification of all plant and animal species by amplification and sequencing information from genes or intergenic target region belonging outside the nuclear genome. A number of authors (Matsuki et al., 2008; Longhi et al., 2009; Galimberti et al., 2014) indicate that molecular-based approaches show great potential to overcome the limitations of previously applied methods. For example, the analysis of certain DNA regions is successfully applied in the identification of plant species in bee pollen (Wilson et al., 2010; Bruni et al., 2012; De Mattia et al., 2012; Parducci et al., 2012; Galimberti et al., 2014). Recent studies indicate that the combination of two plastid regions as genetic markers – rbcL and trnH-psbA are the most effective for determination of plant species in bee pollen gathered by the bees. Galimberti et al. (2014) investigated the effectiveness of the DNA barcode for the characterization of pollen composition. In Bulgaria the melissopalynological analysis are limited (Atanassova et al., 2004; Atanassova and Kondova, 2004; Atanassova et al., 2009; Atanassova et al., 2012) and DNA methods for determination of honey botanical origin are needed. In this respect, the purpose on the present study is to compare two DNA extraction kits – DNeasy Plant Mini Kit (Qiagen) and GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) and CTAB method combined with different pre-treatment procedures and applied to five honey samples.

Materials and Methods

A total of five honey samples were analyzed. Three multifloral honey samples were collected from Bulgarian beekeepers in 2016. The apiaries are located near to Kostinbrod (Sofia region), Kubratovo (Sofia region) and Debelets (Veliko Tarnovo region). Two monofloral honey samples were purchased from the market.

The melissopalynological analysis was carried out using the method established by Bulgarian State Standard for Bee Honey 3050-80 and 2673-80. The pollen analysis was done in the Central Laboratory of Veterinary Control and Ecology, Sofia, Bulgaria.

Two bee pollen samples (BP 1 and BP 2) were used as a control for the procedure for DNA extraction with flash freeze in liquid nitrogen. The samples were multifloral and were collected from the Experimental apiary of the Institute of Animal Science, Kostinbrod by using a pollen trap.

DNA Extraction

Three extraction procedures were employed. Two commercial kits DNeasy Plant Mini Kit (Qiagen) and GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) and CTAB method were used for DNA extraction. Honey (120 g) was diluted with 360 mL MiliQ water, incubated at 45°C for 10 min in a water bath and after that was filtrated through membrane filter (pore size 8 µm). The filter was placed in a sterile petri dish (diameter 5 cm). A volume of 1200 µl of Buffer AP1 from a DNeasy Plant Mini Kit (Qiagen) or 950 µl of Lysis Buffer A (GeneJET Genomic DNA Purification Mini Kit, Thermo Scientific) had been added and shaked for 15 min. After that 400 µL or 350 µL into 3 new tubes (2 mL volume) were transferred. The samples were disrupted using a Tissue Lyser II (Qiagen GmbH) with 3 mm metal beads for 4 min at 30 l/s and then incubated for 10 min at 65°C and 60°C in a water bath. The subsequent steps of the DNeasy Plant Mini Kit were followed according to the manufacturer's instructions, with the exception that the QIAshredder column and second wash stage were omitted. The GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) was followed according to the manufacturer's instructions without any modifications.

The pre-treatment procedures include – sample disruption with a lysis buffer for DNA extraction or grinding the sample in liquid nitrogen at -70°C.

The pollen samples (0.5 g) were flash freezed in liquid nitrogen and after that 1200 µl of Buffer AP1 from a DNeasy Plant Mini Kit (Qiagen) were added. The next steps were the same as for honey samples.

The DNA concentrations of samples were determined by UV spectrophotometry (Nano Drop 2000). The CTAB-based method was performed as described by Murray and Tompson (1980) at 60°C with some modifications (110 mM DTT was added) as described by Lalhmangaihi et al. (2014).

The extracted DNA was stored at -20°C prior to subsequent analysis. Each extraction or amplification method was performed in triplicate.

PCR Amplification

DNA was amplified using the rbcL and trnH-psbA DNA barcode marker region. One of the internationally agreed core DNA barcodes for plants is rbcL whilst trnH-psbA is recognized as valuable additional marker. For PCR amplification of rbcL, the primer combination was 1F: 5'-ATGTCACCACAAACAGAAAC-3' and 724R: 5'-TCGCATGTACCTGCAGTA GC-3'. The primer combination used for trnH-psbA was trnH: 5'-CGCGCATGGTGGATTCACAATCC-3' and psbA: 5'-GTTATGCATGAACGTAATGCTC-3' (Bruni et al., 2015). Conventional PCRs were performed in a total volume of 25 µL. The reaction mixture contained PCR mix Taq polymerase (DNA Polymerase, Bioline), primers, and template DNA solution. Amplification was performed in the Thermal Cycler (Mastercycler-Eppendorf). PCR cycles consisted of an initial denaturation step for 3 min at 94°C, 35 cycles of denaturation (45 s at 94°C), annealing (30 s at 50°C for rbcL and 53°C for trnHpsbA), extension (1 min at 72°C) and a final extension at 72°C for 7 min (Bruni et al., 2015). The DNA extraction and length of amplification products obtained were checked by electrophoresis on 1.8% (w/v) agarose gel and Bioline HyperLadder 100bp. The gel was visualized after being stained with 20 µL GelRed (Biotium) in 200 mL distilled water for 2 hours. The concentration of DNA was check with lambda DNA (50, 25, 12.5, 6.25, 3.13 ng).

The statistical analysis was performed using SPSS Statistical Package, version 21 for Windows. All results are presented as minimal and maximal value, means ± standard deviation.

Results and Discussion

First, the botanical origin of the honey was certified. Pollen analysis was used to specifically identify and confirm the botanical origin of the honey samples. In general, 3 honey samples analyzed could be considered as multifloral and 2 honey samples as monofloral according to pollen contribution of the botanic species. The results are presented in Table 1.

Table 1. Pollen content in the honey samples, (n=5)

Sample	Plant families and species
Sample 1 Multifloral Honey	*Asteraceae* – 44.7±2.3%; (*Helianthus annuus* – 36.9 ±1.9%); *Fabaceae* – 33.0 ±1.7%; (*Melilotus sp.* – 30.0±1.6%); *Brassicaceae* – 3.9 ±0.2%; *Rosaceae* – 2.9 ±0.2%; *Violaceae* (*Viola tricolor* 1.9 ±0.1%); *Poaceae* – 7.8±0.4%; *Betulaceae* – 1.9 ±0.1%; *Chenopodiaceae* – 1.9 ±0.1%.
Sample 2 Multifloral Honey	*Rosaceae* – 65.6 ±3.4%; *Tiliaceae* – 7.9 ±0.4%; *Fabaceae* – 13.2 ±0.7% (*Robinia pseudoacacia* – 5.7 ±0.3%; *Trifolium sp.* – 3.5 ±0.2%; *Melilotus sp.* – 1.3±0.1%); *Asteraceae* – 7.0 ±0.4% (*Helianthus annuus* – 5.3 ±0.3%; *Taraxacum sp.* – 0.88±0.04%); *Poaceae* – 2.2 ±0.1%; *Plantaginaceae* 0.88±0.04%.
Sample 3 Multifloral honey	*Rosaceae* – 31.5 ±1.6%; *Brassicaceae* – 26.8 ±1.4%; *Fabaceae* – 21.4 ±1.4% (*Lotus corniculatus* – 9.5 ±0.5%; *Melilotus sp.* – 5.4 ±0.3%; *Trifolium sp.* – 3.0 ±0.2%); *Tiliaceae* – 7.1 ±0.4%; *Asteraceae* – 4.8 ±0.2% (*Helianthus annuus* – 2.4 ±0.1%).
Sample 4 Sunflower Honey	*Asteraceae* – 81.9 ±4.3% (from them *Helianthus annuus* – 76.1 ±4.0%).
Sample 5 Rape Honey	*Brassicaceae* – 78.5 ±4.1%.

DNA Extraction and Quantitative Analysis

In the present study, we made modifications to select DNA isolation systems to simplify and improve their efficiency in DNA isolation. The yield and quality of isolated DNAs were assessed via gel electrophoresis and spectrophotometric measurement. The DNA isolation from bee honey as a complex matrix of sugars, mineral elements and enzymes has remained very challenging.

1. DNA isolation with DNeasy Plant Mini Kit (Qiagen)

As a first step different pre-treatment procedures were applied for DNA isolation of honey samples with use of only Proteinase K or RNase A. Furthermore, flash freeze in liquid nitrogen was applied for honey sample from Kostinbrod, Kubratovo and Debelets. Two bee pollen samples (P1 and P2) were used as a control. The pretreatment procedure for DNA extraction with flash freeze in liquid nitrogen is suitable but it is not optimal for DNA isolation from honey samples. A further disadvantage of the grinding method with the use of liquid nitrogen is the need to have a continuous source of liquid nitrogen. The results showed that better results are obtained with the both use of Proteinase K and RNase A, without freezing in liquid nitrogen. Figure 1 presents DNA extracted from honey samples with 2 µL Proteinase K (100 mg/mL) and 6 µL RNase A (100 mg/mL). Similar results are obtained for the samples from Debelets and for 2 monofloral honeys (Sunflower and Rape).

2. DNA isolation with GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific)

According to the second method, the results of agarose gel electrophoresis did not show clear visible DNA compare to the result of DNeasy Plant Mini Kit (Qiagen), (Fig. 2). Similar results are obtained for Sunflower and Rape honey samples.

3. CTAB method

The CTAB method is the most widely used method for extracting DNA from plants. The results of agarose gel electrophoresis showed that visible DNA was obtained with CTAB method (Fig. 3) and to a higher extent with the two commercial kits. The CTAB method exhibited the highest DNA intensity for the all honey samples. It was allowed obtaining higher DNA quantity which was particularly evidenced in the studied honey samples.

As can be seen from Fig. 3 (A, B) the CTAB method can extract much more DNA quantity compare to the two commercial kits. Furthermore, the use of CTAB method with common laboratory reagents for DNA extraction instead of commercially available kits make this extraction process more cost effective.

As a next step, the quality of all obtained DNA extracts was assessed by UV spectrophotometry. As shown in Table 2, for each extraction method we have reported the yield and the ratios of spectrophotometric absorbance at 260 and 280 nm. In fact, the ratio A260/280 is usually employed to measure the purity level of DNA and some other nucleic acids and nucleotides. The absorbance A260/280 up to 1.90 generally indicates a “pure” DNA sample. Generally, data were in an agreement with the electrophoretic analysis. As can be expected, the spectrophotometric analyses revealed the presence of DNA with good quality in all samples extracted with DNeasy Plant Mini Kit (Qiagen). Surprisingly, the results of agarose gel electrophoresis of DNA extracted with GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) did not show clear visible DNA. Anyway, spectrophotometric analyses showed similar DNA concentrations (Table 2) but a bit lower ratios of absorbance (A260/280 <1.90) for the most honey samples.

The DNA concentration observed by the CTAB method was higher due to high presence of RNA. The concentration of DNA was additionally checked with lambda DNA (50, 25, 12.5, 6.25, 3.13 ng), (Figure 3A). DNA extracted with the CTAB method from all honey samples showed high concentration. According to the results from the agarose gel and DNA standards it is not more than 3 – 12 ng visible DNA in 5 µL. Similar results for DNeasy Plant Mini Kit (Qiagen) and CTAB method are received by Soares et al. (2015) for honey samples.

PCR Amplification

To evaluate the extracted DNA from the five honey samples with two kits and CTAB method all extracts were amplified by PCR. In the present study, rbcL DNA barcode marker was amplified successfully from the DNA isolated from honey samples (Fig. 4). The amplification results showed strong PCR products (10 µL loaded on agarose gels). As can be seen from Fig. 4 the results obtained after PCR amplification are comparable for the DNA extraction with two commercial kits and CTAB method. With the CTAB method was observed co-precipitation of DNA and yellow pigment which can result in less DNA amplification. The DNA extracted with GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) as shown on Figure 2 is not clear visible. However, it could be easily amplified by PCR demonstrating that the DNA extract is proved adequate and shows strong PCR product in all honey samples (Figure 4).

DNA barcoding analysis was performed with the trnH-psbA intergenic spacer. The trnH-psbA marker was easily amplified in all honey samples.

Figure 5 shows that there is a difference in the number and length of the amplified fragments. Probably, it is due to the better amplification of DNA isolated with GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific) compared to faint and defused bands obtained with DNesay Plant Mini Kit (Qiagen). Furthermore, this was an expected results considering that the multifloral honey samples might contain different pollen grains.

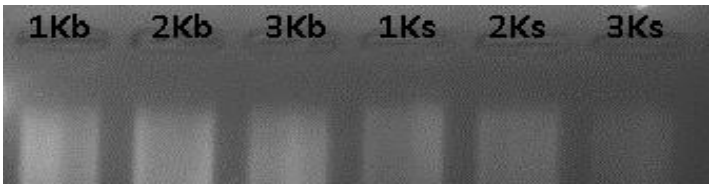


Figure 1. Agarose gel electrophoresis of DNA extracted from honey samples from Kubratovo (1Kb, 2Kb, 3Kb) and Kostinbrod (1Ks, 2Ks, 3Ks), DNeasy Plant Mini Kit (Qiagen)

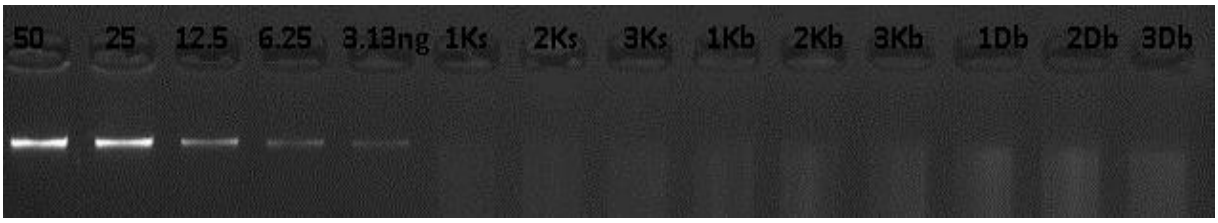
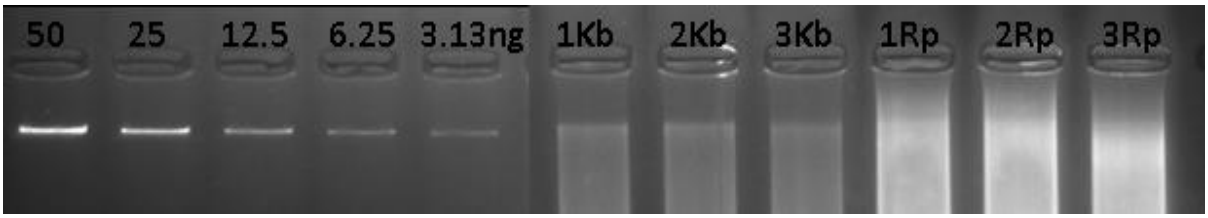
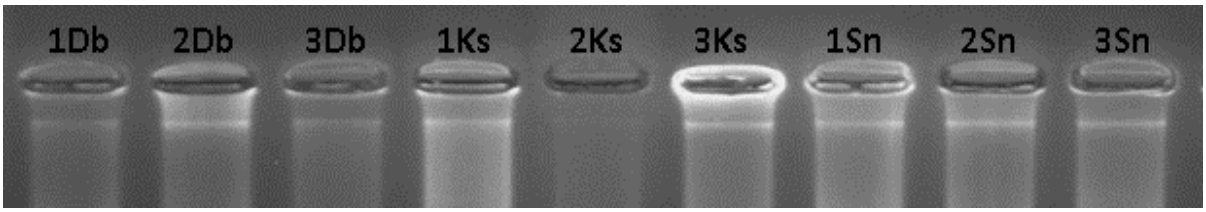


Figure 2. Agarose gel electrophoresis of DNA extracted from honey samples from Kostinbrod (1Ks, 2Ks, 3Ks), Kubratovo (1Kb, 2Kb, 3Kb), Debelets (1Db, 2Db, 3Db), GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific), Lambda DNA (50, 25, 12.5, 6.25, 3.13 ng)

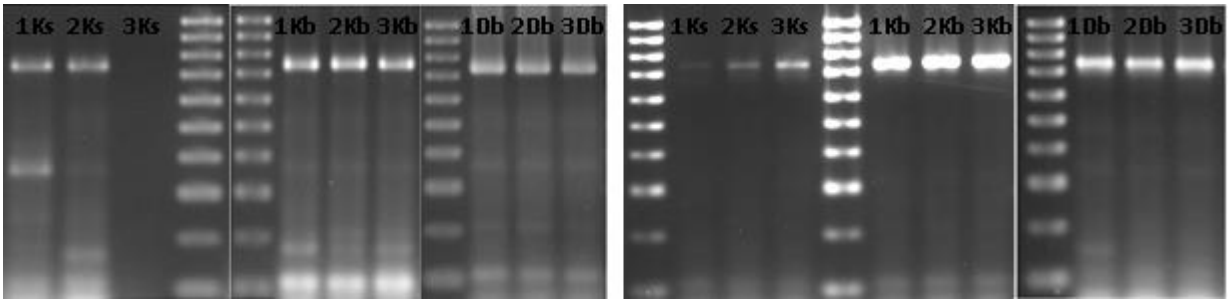


A)

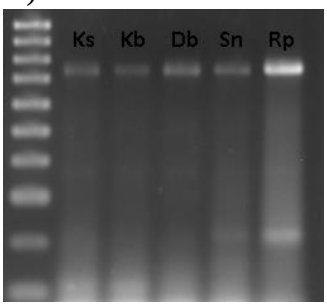


B)

Figure 3. Agarose gel electrophoresis of DNA extracted from honey samples (CTAB method)
A) Lambda DNA (50, 25, 12.5, 6.25, 3.13 ng), Kubratovo (1Kb, 2Kb, 3Kb), Rape honey (1Rp, 2Rp, 3Rp) B) Debelets (1Db, 2Db, 3Db), Kostinbrod (1Ks, 2Ks, 3Ks) and Sunflower honey (1Sn, 2Sn, 3Sn)



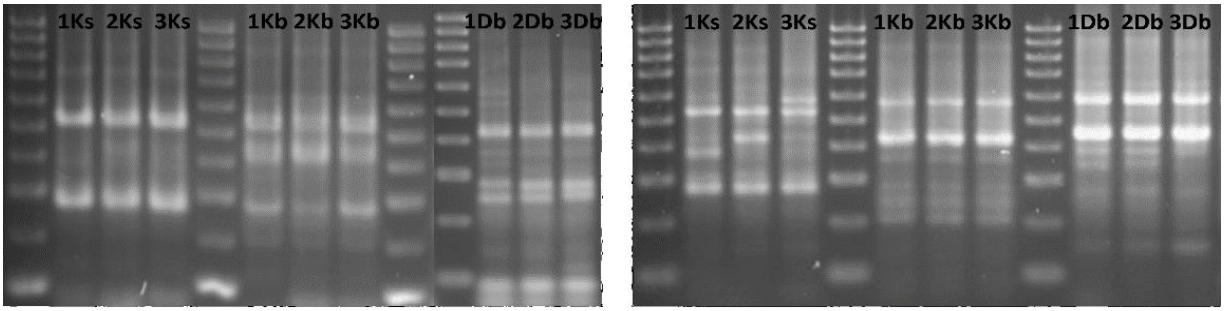
A) B)



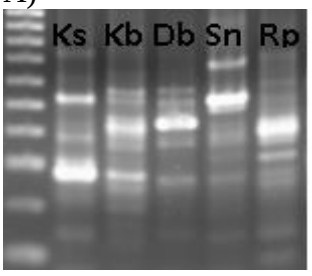
C)

Legend: A) Kostinbrod (1Ks, 2Ks, 3Ks), Kubratovo (1Kb, 2Kb, 3Kb), Debelets (1Db, 2Db, 3Db), DNeasy Plant Mini Kit (Qiagen); B) Kostinbrod (1Ks, 2Ks, 3Ks), Kubratovo (1Kb, 2Kb, 3Kb), Debelets (1Db, 2Db, 3Db), GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific); C) CTAB method – Ks, Kb, Db, Sn, Rp

Figure 4. Agarose gel electrophoresis of PCR products of rbcL DNA barcode marker region amplified from honey samples prepared with two commercial kits and CTAB method



A) B)



C)

Legend: A) Kostinbrod (1Ks, 2Ks, 3Ks), Kubratovo (1Kb, 2Kb, 3Kb), Debelets (1Db, 2Db, 3Db), DNeasy Plant Mini Kit (Qiagen); B) Kostinbrod (1Ks, 2Ks, 3Ks), Kubratovo (1Kb, 2Kb, 3Kb), Debelets (1Db, 2Db, 3Db), GeneJET Genomic DNA Purification Mini Kit (Thermo Scientific); C) CTAB method – Ks, Kb, Db, Sn, Rp

Figure 5. Agarose gel electrophoresis of PCR products of trnH-psbA DNA barcode marker region amplified from honey samples prepared with two commercial kits and CTAB method

The length of the PCR product reflects to the amplification efficiency of our DNA samples. Also, these results are in a good agreement with the pollen analysis. It shows the presence of plant species in which the genetic marker trnH-psbA has a variable and high fragment length ~ 395 and ~ 573 bp. This PCR fragment for *Helianthus annuus* has a length ~ 517 bp, for *Melilotus sp.* ~ 395 bp (in honey sample from Kostinbrod) and for *Robinia pseudoacacia (Fabaceae)* ~ 543 bp, *Rosa canina* 433 bp and *Crataegus monogyna* ~ 394 bp (*Rosaceae*), (in honey sample from Kubratovo and Debelets). On Figure 5 C at lanes 4 (Sn) and 5 (Rp), strong PCR products were observed with ~ 573 bp and ~ 357 bp, respectively. It can be assumed that this marker can be used in studies including molecular identification of botanical origin of monofloral honey samples.

According to Bruni et al. (2015) the plastid spacer trnH-psbA was the most suitable marker to determine the plant species composition of honey. Furthermore, the DNA analysis is faster than melissopalynological analysis and can be used for routine use.

Conclusion

This study describes for the first time DNA extraction from Bulgarian multifloral and monofloral honey samples. According to the results obtained, we can concluded that all tested DNA isolation procedures and commercial kits presented, result in isolation of sufficient amount of pollen DNA from multifloral and monofloral honey samples. Thus, extracted DNA is successfully used for PCR amplification of rbcL gene and trnH-psbA spacer regions. The obtained results will be in a further use for routine application of PCR based molecular methods for identification and verification of botanical origin of the honey or determining the presence or absence of plant species.

Acknowledgments

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References

Atanassova, J., Bozilova, E., Todorova, S. (2004). Pollen analysis of honey from the region of three villages in West Bulgaria. *Phytologia Balcanica*, 10(2-3), 247-252.

Atanassova, J., Kondova, V. (2004). Pollen and chemical-physical analysis of unifloral honey from different regions of Bulgaria. *Phytologia Balcanica*, 10(1), 45-50.

Atanassova, J., Yurukova, L., & Lazarova, M. (2009). Palynological, physical, and chemical data on honey from the Kazanlak region (Central Bulgaria). *Phytologia Balcanica*, 15(1), 107-114.

Atanassova, J., Yurukova, L., Lazarova, M. (2012). Pollen and inorganic characteristics of Bulgarian unifloral honeys. *Czech Journal of Food Sciences*, 30(6), 520-526.

Bruni, I., De Mattia, F., Martellos, S., Galimberti, A., Savadori, P. Casiraghi, M., Nimis, P.L., Labra, M. (2012). DNA Barcoding as an effective tool in improving a digital plant identification system: A case study for the area of Mt. Valerio, Trieste (NE Italy). PLoS ONE 7(9): e43256.

Bruni, I., Galimberti, A., Caridi, L., Scaccabarozzi, D., De Mattia, F. (2015). A DNA barcoding approach to identify plant species in multiflower honey. *Food Chemistry*, 170(1), 308-315.

Bulgarian State Standard (1980). Rules for sampling and tasting methods. Qualitative indicators on honey 3050-80. http://www.bds-bg.org/bg/standard/?natstandard_document_id=61915 (accessed 18.10.2017)

Bulgarian State Standard (1980). Bee Honey 2673-80. De Mattia, F., Gentili, R., Bruni, I., Galimberti, A., Sgorbati S. (2012). A multi-marker DNA barcoding approach to save time and resources in vegetation surveys. *Botanical Journal of the Linnean Society*, 169, 518–529.

Fernandez-Torres, R., Pérez-Bernal, J., Bello-Lopez, M., Callejón-Mochón, M., Jimenez-Sanchez, J., Guiraum-Perez, A. (2005). Mineral content and botanical origin of Spanish honeys. *Talanta*, 65, 686-691.

Galimberti, A., De Mattia, F., Bruni, I., Scaccabarozzi, D., Sandionigi, A., Barbuto, M., Casiraghi, M., Labra, M. (2014). A DNA barcoding approach to characterize pollen collected by honeybees. PLoS ONE 9(10): e109363.

Guyot, C., Scheirman, V., Collin, S. (1999). Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*. *Food Chemistry*, 64, 3-11.

Hernandez, O.M., Fraga, J.M.G., Jiménez, A.I., Arias, J.J. (2005). Characterization of honey from the Canary Islands: Determination of the mineral content by atomic absorption spectrophotometry. *Food Chemistry*, 93, 449-458.

Lalhmangaihi, R., Ghatak, S., Laha, R., Gurusubramanian, G., Kumar, N. (2014). Protocol for optimal Quality and Quantity Pollen DNA isolation from Honey samples. *Journal of Biomolecular Thechniques*, 25, 92-95.

Latorre, M.J., Pena, R., Pita, C., Botana, A., Garcia, S., Herrero, C. (1999). Chemometric classification of honeys according to their type. II. Metal content data. *Food Chemistry*, 66, 263-268.

Liberato, M., Morais, S., Magalhaes, C., Magalhaes, I., Cavalcanti, D., Silva, M. (2013). Physicochemical properties and mineral and protein content of honey samples from Ceará State, Northeastern Brazil. Food Science and Technology (Campinas), 33(1), 38-46.

Longhi, S., Cristofori, A., Gatto, P., Cristofolini, F., Grando, M.S., Gottardini, E. (2009). Biomolecular identification of allergenic pollen: A new perspective for aerobiological monitoring. *Annals of Allergy, Asthma and Immunology*, 103, 508-514.

Matsuki, Y.R., Tateno, R., Shibata, M., Isagi, Y. (2008). Pollination efficiencies of flowervisiting insects as determined by direct genetic analysis of pollen origin. *American Journal of Botany*, 95, 925-930.

Murray, H.G., Tompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids Research*, 8(19), 4321-4325.

Parducci, L., Matetovici, I., Fontana, S.L., Bennet, K.D.Y., Suyama, Y., Haile, J., Kurt, H., Kjaer, K.H., Larsen, N., Drouzas, A., Willerslev, E. (2012). Molecular and pollen-based vegetation analysis in lake sediments from Central Scandinavia. *Molecular Ecology*, 22, 3511-3524.

Radovic, B.S., Careri, M., Mangia, A., Musci, M., Gerboles, M., Anklam, E. 2001. Contribution of dynamic head space GC–MS analysis of aroma compounds to authenticity testing of honey. *Food Chemistry*, 72, 511-520.

Soares, S., Amaral, J.S., Oliveira, M.B.P.P., Mafra, I. (2015). Improving DNA isolation from honey for the botanical origin identification. *Food Control*, 48, 130-136.

Soria, A.C., Gonzalez, M., De Lorenzo, C., Martínez-Castro, I., Sanz, J. (2004). Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopaynological, physicochemical and volatile data. *Food Chemistry*, 85, 121-130.

Wang, J., Li, Q.X. (2011). Chemical composition, characterization, and differentiation of honey botanical and geographical origins. *Advances in Food and Nutrition Research*, 62, 89-137.

Wilson, E.E., Sidhu, C.S., Le Van, K.E., Holway, D.A. (2010). Pollen foraging behavior of solitary Hawaiian bees revealed through molecular pollen analysis. *Molecular Ecology*, 19, 4823-4829.

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GIDA BİLEŞENLERİNİN SPREY SOĞUTMA YÖNTEMİ İLE ENKAPSÜLASYONU

Emine Varhan, Mehmet Koç

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Adnan Menderes Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, 09010 Aydın, Türkiye

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Correspondence:

Mehmet KOÇ

E-mail: mehmetkoc@adu.edu.tr

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ÖZ

Sprey soğutma yöntemi ile gıda bileşenlerinin enkapsülasyonu son yıllarda dikkat çeken bir teknoloji olup, özellikle ısıya ve suya duyarlı aktif maddelerin kaplanmasında kullanılmaktadır. Sprey soğutma yöntemi, aktif maddenin kaplama materyali veya materyalleri içerisine dispersiyonu, bu karışımın atomizasyonu ve katılaştırma aşamalarından oluşmaktadır. Sprey soğutma yöntemi ile enkapsülasyon işleminde mumlar, hidrojenize yağlar, yağ asitleri ve düşük sıcaklıklarda jel oluşturma özelliği iyi olan protein ve karbonhidratlar kaplama materyalleri olarak kullanılmaktadır. Kaplama materyallerinin tipi ve karışım oranı diğer enkapsülasyon yöntemlerinde olduğu gibi sprey soğutma yönteminde de enkapsülasyon etkinliğini belirleyen önemli özelliklerdir. Sprey soğutma yönteminin kullanıldığı çalışmalarda genellikle aromalar, vitaminler, mineraller, yağlar ve probiyotikler enkapsüle edilmiştir. Bu çalışmalarda kullanılan kaplama materyallerinin çeşidinin ve oranının enkapsülasyon etkinliği üzerine etkisi ve/veya depolama süresince enkapsüle edilen aktif maddenin stabilitesi incelenmiştir. Bu derleme çalışmasında, sprey soğutma yönteminin enkapsülasyon işleminde kullanılabilirliği ve literatürde gerçekleştirilen çalışmaların kapsamları detaylı olarak değerlendirilmiştir.

Anahtar Kelimeler: Sprey soğutma, Enkapsülasyon, Hidrojenize yağlar, Vitaminler, Enkapsülasyon etkinliği

ABSTRACT

ENCAPSULATION OF FOOD MATERIALS WITH SPRAY COOLING METHOD

Encapsulation of food materials with spray cooling has been a remarkable technology in recent years and is especially used for encapsulating heat and water sensitive active materials. The spray cooling method comprises the dispersion of the active material into the coating material or materials, the atomization of the mixture and the solidification stages. In the food industry, aromas, vitamins, oils and probiotics can be encapsulated by using spray cooling method. Waxes, hydrogenated oils, fatty acids and, proteins and carbohydrates that are good at forming gels at low temperatures are used as coating materials in the encapsulation process by spray cooling. The type and mixing ratio of coating materials are the most important features that determine the encapsulation efficiency in spray cooling as well as in other encapsulation methods. Aromas, vitamins, minerals, oils and prebiotics are mostly encapsulated in the studies where spray cooling method is used. In these studies, the effect of coating material type and ratio on encapsulation efficiency and/or the storage stability of active material have been investigated. In this review study, usability of spray cooling method in encapsulation process and the scope of the studies carried out in the literature have been evaluated.

Keywords: Spray cooling, Encapsulation, Hydrogenated oil, Vitamins, Encapsulation efficiency

Giriş

Gıda tüketim tercihlerinin ve davranışlarının değişime uğraması sonucunda, sağlıklı beslenme ve hazır gıda kavramları birbirleriyle bağlantılı olarak her geçen gün önem kazanmaktadır. Günümüzde hızlı yaşam koşullarına uygun, hazır ve kolay tüketilebilir gıdaların talebi doğrultusunda değişen tüketici istekleri dikkate alınarak, gıda sanayi hazır gıda ve farmasötik ürünler üzerinde yenilikçi bir vizyonla çalışmalarını yürütmektedir (Zungur, 2013). Enkapsülasyon teknolojisi de fonksiyonel ve spesifik gıda talebi doğrultusunda gıdanın türüne bağlı olarak gelişim göstermekte ve gıda içerisindeki aktif bileşenin korunumu, tat ve kokusunun maskelenmesi, hedeflenen doğrultuda kontrollü salınım ve kolay taşınıp, depolanması gibi yönleriyle fayda sağlamaktadır.

Enkapsülasyon; bir maddenin veya karışımın başka bir madde ile kaplanması veya hapsedilmesi olarak tanımlanmakta ve elde edilen ürün boyutuna bağlı olarak makro, mikro veya nano ön ekleri alarak isimlendirilmektedir (Koç vd., 2010).

Enkapsülasyon işleminde nişasta, maltodekstrin, pullulan, sakkaroz, maltoz gibi karbonhidratlar; jelatin, peynir altı suyu proteinleri, kazein, kazeinatlar gibi proteinler ve gamlar (Koç vd., 2010) tercih edilerek genellikle kabuk, duvar, membran, taşıyıcı veya kapsül oluşturucu olarak adlandırılmaktadırlar. Bu kaplama materyalleri ile kaplanacak olan hassas malzemeye (katı ve sıvı yağlar, aroma ve tat bileşenleri, vitaminler, mineraller, renk bileşenleri ve enzimler) ise iç faz, çekirdek veya aktif madde adı verilir (Sagis, 2015).

Enkapsülasyon uygulaması başta gıda olmak üzere, tarım, ilaç, enerji ve savunma gibi alanlarda kullanılmakta olup, temel amaçları:

- Uyumsuz bileşikleri ayırma,
- Sıvı materyalin katı hale getirilmesi,
- Olumsuz çevre koşullarına karşı stabiliteyi arttırma (oksidasyon ve deaktivasyona karşı mikroenkapsüle materyali korumak),
- Mikroenkapsüle edilen materyalin tat ve kokusunun maskelenmesi,
- Aktif bileşiklerin uçuculuğunun önlenmesi,
- Mikroenkapsüle materyallerin hedeflendiği şekilde kontrollü olarak salınımının sağlanması,
- Kaplanan çekirdek materyalin diğer bileşenlerle reaksiyonlara girmesinin önlenmesi,
- Mevcut çevrenin korunması,
- Malzemenin daha kolay taşınması ve depolanması şeklinde açıklanabilinir (Ünal ve Erginkaya, 2010; Sagis, 2015).

Fonksiyonel ve spesifik gıda üretimi için bir çok gıdanın enkapsülasyonunda, gıdanın türüne bağlı olarak sprey kurutma, sprey soğutma, dondurarak kurutma, akışkan yatak kaplama, ekstrüzyon, koaservasyon, kokristalizasyon ve lipozom tutuklama gibi çeşitli metotlar tercih edilerek, kullanılabilinir (Koç vd., 2010; Alvim vd., 2016).

Bu derlemede jel mikropartikül üretimine uygun ve ucuz teknolojik bir yöntem olduğu düşünülen sprey (püskürtmeli) soğutma yöntemi üzerinde durularak, genel itibariyle enkapsüle edilecek çekirdek materyalini oluşturan bileşik yapısına etkisi hakkında bilgi sunulacaktır.

Sprey Soğutma Yöntemi ile Enkapsülasyon

Gıda maddelerinin enkapsülasyonu üzerine son zamanlarda çok fazla çalışma yapılsa da halen enkapsülasyon yöntemi ve işlem değişkenlerinin son ürün üzerine olan etkisi incelenmelidir. Artan gıda ürünlerinin karmaşıklığı, araştırmaları yeni ve farklı kapsülleme teknikleri ve süreçlerine yönlendirmeye devam etmekte ve özellikle fonksiyonel gıda tüketiminin artmasıyla beraber bu ürünlerde kullanılan aktif bileşenlerin korunumu üzerine çalışmalar hız kazanmaktadır. Mikrokapsül yapılarının hazırlanmasına yönelik kullanılan yöntemler; fiziksel (sprey kurutma, sprey soğutma, dondurarak kurutma, ekstrüzyon, sıvı boncuk kaplama vs.), kimyasal (moleküler inklüzyon kompleksasyonu ve ara yüzey polimerizasyonu) ve fizikokimyasal (koaservasyon, lipozom tutuklama vb.) olarak sınıflandırılabilir (Koç vd., 2010).

Sprey soğutma, diğer yöntemlere kıyasla benzersiz bir matris serbest bırakma mekanizması ile dengeli farmasötik kapsüllerin üretiminde yıllardır kullanılan alternatif bir teknik olup, suya duyarlı aktif maddeleri kapsüllemek için solventsiz bir enkapsülasyon yöntemidir (Lakkis, 2007). Genel olarak sprey soğutma yöntemlerinde izlenen üç temel ve ortak ilke sırasıyla; kaplanacak olan etken maddenin matris malzemesine dispersiyonu, elde edilen karışımın atomizasyonu ve matris malzemesinin katılaştırılması işlemlerine dayanmaktadır (Garti ve McClements, 2012).

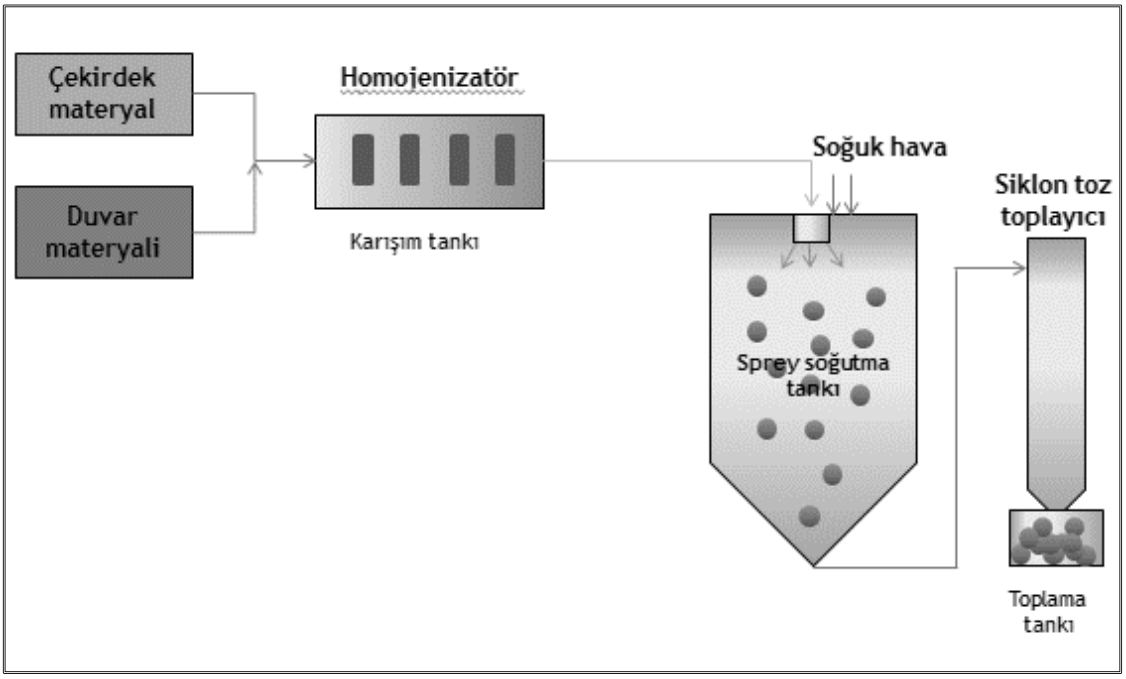
Şekil 1'de görüldüğü üzere sprey soğutma işleminde enkapsülasyon yapısının oluşturulabilmesi amacıyla çekirdek materyali, atomizasyon basamağından önce kaplama

materyali olarak kullanılacak matris malzemesi içerisine dağıtılır; atomizasyon ve soğutma işlemi sonrası matris, bir mikroküre veya çok çekirdekli bir mikrokapsül oluşturmak üzere dağılmış olan aktif bileşen etrafında katılaşır (Garti ve McClements, 2012). Yağlı kaplama olarak da bilinen sprey soğutma yöntemi atomizasyon kaynağı, parçacık oluşturma odası ve toplama bölgesini içermesi bakımından birçok yönüyle sprey kurutma işlemiyle benzerdir. Fakat kaplama materyalinin çekirdek materyal etrafını sarmalayarak katılaşmasını sağlayan soğutulmuş havaya atomize edilmesi ile sprey kurutma yönteminden ayrılmaktadır. Ayrıca sprey soğutma yöntemi ile enkapsülasyon işlemi, sprey kurutma yöntemindeki gibi suyun buharlaştırılması basamağını içermediğinden dolayı atomizasyon işlemi sonrası oluşan sıvı damlacıkların iç kısmında yer alan çekirdek materyali duvar materyallerinin katılaşması sonucunda hapsedilir (Rokka ve Rantamaki, 2010; Garti ve McClements, 2012).

Sprey soğutma yöntemi, diğer uygulamalara nazaran daha küçük partikül boyutlarında başarılı bir şekilde elde edilen mikrokapsül yapılarının, lipit kaplamaya bağlı olarak suda çözünmemesi dolayısıyla; gıda bileşenleri ve nutrasötik uygulamalar için mineral maddeler, probiyotikler, suda çözünen vitaminler, enzimler, asitleştiriciler, aroma bileşenleri, besleyici yağ ve biyoaktif maddelerinin enkapsülasyonunda yaygın olarak tercih edilen basit bir yöntemdir (Rokka ve Rantamaki, 2010; Desai ve Park, 2005; Garti ve McClements, 2012). Sprey soğutuma yönteminde materyal içerisindeki suyun giderimi sağlanmadığı için kütle transferi gerçekleşmez. Bu nedenle atomize hale geçen damlacık yapılarının soğuk havayla teması sonrası, kusursuz küresel şekiller halinde katılaşan, serbestçe akan toz parcacıkları elde edilir (Desai ve Park, 2005).

Sprey soğutma yöntemi ile enkapsülasyon işleminde sıklıkla tercih edilen kaplama materyalleri genellikle bitkisel yağ ya da türevleridir. Düşük erime noktasına (32-42 °C) sahip parçalanmış veya hidrojene edilmiş bitkisel yağların yanı sıra 45-122 °C erime noktalarına sahip sıkı mono ve diasilgliseroller gibi çok çeşitli kaplama materyalleri sprey soğutma yöntemi ile enkapsülasyon işleminde kullanılmaktadır (Desai ve Park, 2005).

Sprey soğutma yöntemi ile enkapsülasyon işleminin diğer yöntemlere kıyasla sağlamış olduğu avantajlar; yüksek ısı uygulamasının olmaması, düşük işletim maliyeti ve solventsiz çalışılmasıdır. Ayrıca kaplama materyali olarak lipid türevi bileşiklerin kullanımı sprey soğutma yöntemi sonucu elde edilen enkapsüle toz ürünlere yüksek nem direnci sağlaması bakımından karşılaştırıldığında; suda çözünür amorf polisakkaritlere dayalı sistemlere kıyasla da avantajlar sağladığı görülmektedir. Fakat bahsedilen avantajların yanı sıra oksijen difüzyonuna karşı kristallerin apolar yapılarının zayıf bir bariyer özelliği göstermesi gibi dezavantajları göz önünde bulundurmak gerekmektedir (Sartori vd., 2015; Matos-Jr vd., 2017).



Şekil 1. Sprey soğutma yöntemiyle enkapsülasyon işleminin akış diagram

Figure 1. Flow diagram of encapsulation with spray cooling method

Dispersiyon Hazırlama

Sprey soğutma yöntemi ile enkapsülasyon işleminin birinci basamağı olan dispersiyon hazırlama aşamasında kaplanacak olan aktif bileşen kaplama materyallerini içeren matris ortamı içerisine disperse edilerek homojen bir dağılım sağlanmaya çalışılmaktadır. Mikroenkapsüle edilmek istenen çekirdek malzeme, sıvı veya katı olabileceği gibi matris malzemesi ile karışabilir veya karışmaz bir yapıda olabilir. Atomizasyon işlemi öncesi, kaplama materyali olarak da adlandırılan matris malzemesi ve aktif bileşen içeren bir besleme dispersiyonu hazırlanır (Garti ve McClements, 2012). Hazırlanan besleme dispersiyonunun özellikleri, kaplama malzemesinin türü ve yoğunluğuna ve dispersiyonun besleme sıcaklığına bağlı olarak değişkenlik gösterebilir (Sagis, 2015). Aktif madde dediğimiz çekirdek materyalin karışabilir özellikte olması halinde matris materyalinde çözünmesi, karışmaması durumunda ise emülsifikasyonu sağlanırken, emülsiyonlaştırmanın gerekli olduğu durumda yüzey aktif bileşenleri stabil bir emülsiyon oluşturmak için kullanılabilinir (Garti ve McClements, 2012).

Matris malzemesi içerisinde çözünmeyen bileşenlerin sprey soğutma yöntemi ile enkapsülasyonunda, son üründeki enkapsülasyon etkinliği hazırlanan emülsiyonun stabilitesinden etkilenmekte olup, stabilite iyi olduğunda enkapsülasyon etkinliği artmaktadır (Zungur vd., 2013).

Kaplama Materyalleri

Enkapsülasyon işleminin etkinliği kaplama materyallerine büyük oranda bağlıdır. Enkapsülasyon işlemi için kullanılan kaplama materyalleri genel anlamıyla lipofilik ya da hidrofilik olarak da sınıflandırılabilir. Enkapsülasyon işlemleri için işlevsel lipofilik veya hidrofilik yapıların oluşturulabilmesi için çeşitli kaplama materyalleri kullanılabilir (Kwak, 2014).

İdeal bir kaplama materyali:

- Yüksek konsantrasyonda reolojik özellikleri iyi olmalı ve kapsülleme işlemi esnasında kolay işlenebilmelidir.
- Emülsiyon ve dispersiyon özelliğine sahip olmalı ve emülsiyon stabilitesi yüksek olmalıdır.
- Kaplama işlemi esnasında ve/veya depolama sırasında çekirdek materyalin özelliğini bozacak şekilde reaksiyona girmemelidir.
- Çekirdek materyalini kaplayabilmeli ve bunu stabil bir şekilde hem işlem esnasında hem de depolama esnasında koruyabilmelidir.
- İstenilen çözgende çözünebilmeli ve maliyet açısından uygun olmalıdır (Koç vd., 2010).

Mikroenkapsulasyon işleminde genellikle nişasta, maltodekstrin, pullulan, sakkaroz, maltoz gibi karbonhidratlar; jelatin, peynir altı suyu proteinleri, kazein ve kazeinatlar gibi proteinler ve gam arabik gibi gamlar kaplama materyali olarak tercih edilmekte olup, probiyotik bakterilerin enkapsülasyonunda yaygın olarak kullanılan kaplama materyalleri yosun orijinli polisakkaritler (karrageenan, aljinat), bitkiler (nişasta ve türevleri, Arap gamı) ve bakterilerden (gellan, ksantan) ve hayvansal proteinlerden (süt, jelatin) elde edilir (Rokka ve Rantamaki, 2010).

Sprey soğutma yöntemiyle enkapsülasyon işleminde ise kaplamanın çekirdek materyali etrafında katılaşması, eriyik haldeki karışımın serin bir hava akımına püskürtülmesi şeklinde gerçekleşir. Kaplama materyalleri eriyik halde sıvı formda iken oda sıcaklığında katılaşabilmelidir. Bu nedenle sprey soğutma yöntemi ile enkasülasyon işleminde yüksek sıcaklıkta eriyen polimerler, mumlar, hidrojenize yağlar ve yağ asitleri gibi lipit türevleri kullanılmaktadır (Kwak, 2014). Ayrıca düşük sıcaklıklarda jel oluşturma özelliği iyi olan protein ve karbonhidratlar da kaplama materyali olarak tercih edilmektedirler.

Lipitler

Sprey soğutma yöntemi ile enkapsülasyon yönteminde kaplama materyali olarak kullanılan yağlar laurik, palmitik ve oleik-linoleik gruplar gibi üç temel yağ asidi grubunun bir veya daha fazla formülasyonlarından oluşmaktadırlar. Bu yağ asitlerinin karboksilik asit zincir uzunluklarındaki değişiklikler, erime profilleri, doygunluk derecesi, esterifikasyon derecesi, saflık dereceleri ve bunların kristalimsi yapısı kaplamanın işlenebilirliğinin yanı sıra kapsüllenmiş ürünün performansı üzerinde önemli bir etkiye sahiptir (Lakkis, 2007). Kaplama materyali olarak kullanılacak olan yağların erime noktasının düşmesi ile birlikte sprey soğutma işlemi sonrası elde edilen ürünün kümeleşme eğilimi yüksek, akışkanlığı ise düşüktür.

Oleik / linoleik asit grubu: mısır, pamuk tohumu, yer fıstığı, zeytin, ayçiçeği, aspir gibi ticari öneme sahip bu yağlar farklı sertlik derecelerinde plastik yapıdaki yağlar oluşturmak üzere hidrojene edilebilir. Sprey soğutma yöntemi ile enkapsülasyon uygulamalarında bu yağ asitlerinin sadece yüksek seviyede hidrojenlenmiş versiyonları etkili olmaktadır (Lakkis, 2007). Ribeiro vd. (2012) sprey soğutma yöntemi ile glikoz içeren stearik asit mikropartiküllerini oluşturmak için oleik asit içerikli kaplama materyali kullanmışlardır. Sartori vd. (2015) ise askorbik

asidin oleik asit içerikli kaplama materyalleriyle sprey soğutma işlemi gerçekleştirerek mikrokapsülasyon verimi ve yapısını incelemişlerdir.

Laurik asit grubu: Bu grubun yağ asitleri doymuş, kısa zincirli yağ asitleri bakımından zengin (8, 10 ve 14 karbon zincir uzunluğu) ve kararlı olup, ortalama % 40-50 oranında laurik asit içerirler. Doymamış fraksiyonların çoğunluğunu oleik ve linoleik asitler oluştururken doymuş olanlar esas olarak palmitik ve stearik asitlerden oluşmaktadır. Laurik asit esaslı yağ asitleri nispeten düşük erime noktalarına (~44°C) sahiptir. Sartori vd. (2015) farklı Laurik asit/Oleik asit oranlarınındaki kaplama materyallerinin proses verimi üzerine etkilerini incelemiş; kaplama malzemesi içerisindeki laurik asit miktarının çekirdek materyal salınımını araştırmışlardır.

Palmitik asit grubu: Palm yağı % 32-47 palmitik asit ve % 40-52 oleik asit içermekte olup bu yağ asidi grubu, doymuş ve doymamış yağ asitlerinin eşit konsantrasyonlarına sahiptir. Matos-Jr vd. (2015) hidrojene palm yağı ve bitkisel gliserol monostearat kaplama materyallerini kullanarak, sprey soğutma yöntemi ile askorbik asit içeren mikroparçacıklarının; verim, morfoloji, ortalama boyut ve dağılımı, termal davranışları, kapsülleme verimliliği ve askorbik asit kararlılığı açısından karakterize edilmesi üzerine çalışma yürütmüşlerdir. Ayrıca Matos-Jr vd. (2017) askorbik asidin sprey soğutma yöntemi ile kapsüllenmesinde kaplama materyali olarak palm ve palm çekirdeği yağı; emülsifiyer olarak soya lesitini ile farklı besleme formları ve karıştırma hızlarında denemeler gerçekleştirmişlerdir.

Mumlar: Doğal ve sentetik mumlar sprey soğutma yöntemi ile enkapsülasyon işleminde yüksek erime sıcaklıklarına sahip olmalarından dolayı kullanılmakta olup, en sık kullanılan mumlar karnauba, kandelila ve bal mumudur.

Karnauba mumu yüksek erime noktasına (82-86°C) ve özgül ağırlığına sahip olması nedeniyle erime noktası, sertlik, tokluk ve parlaklığı artırmak için diğer mumlarla birlikte gıda sistemlerinde spesifik uygulamalar için kullanılabilinir (Lakkis, 2007). Sprey soğutma yöntemi ile enkapsülasyon işleminde kullanılan diğer bir mum çeşidi olan bal mumu 61-65°C erime sıcaklığına sahiptir ve GRAS olarak kabul edilmesi nedeniyle doğrudan kullanıma izin verilmektedir (Lakkis, 2007). Bal mumunun enkapsülasyon işlemlerinde donuk halde kırılganlığının çok kolay olması tek başına kullanımından ziyade farklı kaplama materyalleri ile kombinasyonlarının yapılmasını gerektirmektedir. Sertlik derecesi bakımından balmumu ile karnauba arasında olan candelilla, az miktarda ester ve serbest yağ asidi içerir. Candelilla da bal mumu gibi GRAS olarak kabul edilir ve bazı gıda kullanımları için izin verilir (Lakkis, 2007).

Proteinler ve Karbonhidratlar

Lezzet bileşenlerinin bağlanmasında oldukça iyi olan proteinler sahip oldukları fonksiyonel özelliklerinden dolayı mikroenkapsülasyon işlemi için iyi bir kaplama materyalleridir. Genellikle mikroenkapsulasyon işleminde kaplama materyali olarak jelatin, peynir altı suyu proteinleri, kazein ve kazeinatlar tercih edilmektedir (Koç vd., 2010). Peynir altı suyu proteini gibi nano taşıyıcı sistemin geliştirilmesiyle fonksiyonel bileşiklerin biyolojik olarak kullanılabilirlikleri büyük ölçüde artmış, vitamin ve minerallerin mukozal sistemlere ulaşmasına da imkan sağlamıştır (Kwak, 2014).

Hidrojel yapısını sağlayan jelatin, karagenan, gellan gam, pektin, alginat, agar gibi bazı protein ve karbonhidratlar sprey soğutma işleminde atomize damlacıkların katılaşmasının sağlanmasında, jelatinasyon sıcaklığına dayanarak kapsülleme yapılabilmesi için yumuşak sulu bir matris sistemi sunar. Besleme solüsyonu genel olarak su, hidrokolloid, jelleştirme ajanı ve aktif bileşenden oluşmakta olup, ek olarak hacim arttırıcı maddeler, surfektanlar, antioksidanlar ve plastikleştiriciler şeklinde besleme solüsyonunun iyileştirilmesinde kullanılabilirler (Garti ve McClements, 2012).

Literatürde proteinler ve protein hidrolizatlarının sprey soğutma kullanılarak kapsüllendiği çalışmalarda temel amaç, acı tadı maskelemek ve protein hidrolizatlarının gıdalarda istenmeyen değişikliklere sebep olan yüksek reaktifliğini bastırmak olmuştur. Yajima vd. (1999) güçlü acı aromaya sahip bir antibiyotik olan klaritromisinin sprey soğutma ile mikroenkapsülasyonu üzerinde çalışmışlar, araştırıcılar klaritromisinin mikroenkapsüle edildikten sonra hem yüksek biyoaktifliğe sahip olduğunu hem de sindiriminin ağızda değil, bağırsaklarda gerçekleştiğini rapor etmişlerdir.

Dispersiyonun Atomizasyonu

Sprey soğutma yöntemi ile enkapsülasyon işleminde kararlı bir süspansiyon veya emülsiyon hazırlandıktan sonra karışım bir atomizöre pompalanır, uniform ve birbirinden ayrı küçük damlacık yapıları üretilir. Atomizasyon işlemi sprey kurutma yönteminde olduğu gibi çift akışkanlı nozul, döner atomizör, disk atomizör, basınçlı nozul veya ultrasonik nozul ile gerçekleştirilir (Garti ve McClements, 2012).

Gıda Endüstrisinde Sprey Soğutma Yöntemi ile Üretilmiş Ürünler

Sprey soğutma yöntemi ile enkapsülasyon işlemi, tat maskeleme, kontrollü salınım, sıvının katı bir yapı kazanması, çevresel etkilerden koruma, aktif bileşenlerin ayrılması ve/veya biyoyararlanımda artış gibi nedenlerden dolayı tercih edilmektedir. Tablo 1'de özetlendiği üzere, gıda endüstrisinde aromalar, vitaminler, mineraller, yağlar ve probiyotikler gibi bileşenlerin kaplanmasında sprey soğutma yönteminin kullanıldığı görülmektedir.

Aroma Uygulamaları

Aroma bileşenlerinin çözünürlüğünün farklı olması nedeniyle hem jel hem de eriyik matris sistemleri kullanılarak sprey soğutma yöntemi ile enkapsülasyon işlemi gerçekleştirilebilir. Bir çok aroma karışımının uçucu olmaları nedeniyle sıcaklığının çok yüksek olduğu durumlarda kayıplar daha yüksek olacaktır. Termal kayıpların önemsenmediği bazı aromatik yağlarda eriyik malzemeler kapsülleme matrisi olarak kullanılabilinirken, bazı aromatik bileşenlerindeki termal kayıpların önüne geçebilmek için ise jeller kullanılabilir (Garti ve McClements, 2012). Zencefil oleoresin antimikrobiyal ve antioksidan özellikleri ile bilinmesinin yanısıra aromatik bir bileşen olarak sıklıkla kullanılmaktadır. Oriani vd. (2016), zencefil oleoresin yüklü, katı lipid parçacıklarının sprey soğutma tekniği ile oluşumu ve karakterizasyonununu inceledikleri çalışmalarında farklı erime sıcaklıklarına sahip palm yağlarını kaplama materyali olarak kullanmışlardır. Erime sıcaklığının yüksek olduğu palm yağının kullanıldığı durumda, daha organize bir kristal yapının oluşması nedeniyle zencefil oleoresinin enkapsülasyonu için daha etkili olduğunu bulgulamışlardır.

Nutrasötik Uygulamalar

Antioksidanlar, vitaminler, elzem yağ asitleri, proteinler ve enzimler gibi nutrasötiklerin kapsüllenmesi çeşitli kaplama materyalleri kullanılarak, farklı yöntemler ile gerçekleştirilmekte olup, literatür incelemeleri sonucunda bazı nutrasötiklerin sprey soğutma yöntemi ile enkapsüle edilmesi üzerine yapılan/devam eden başarılı çalışmalar bulunmaktadır.

Kwak (2014) C vitamininin işlenmesi ve stabilize edilebilmesi için sprey soğutma yöntemi ile mikroenkapsüllenmesini inceleyerek, kaplama materyali olarak kullanılan yağ asitleri karışımındaki oleik asit içeriğinin, kaplama materyali ile çekirdek materyalin arasındaki oran dikkate alınmaksızın, katı lipit mikrokapsüllerinin kapsülleme kapasitesini olumlu yönde etkilediğini gözlemlemişlerdir. Sartori vd. (2015) C vitamini mikroenkapsülasyonu için yapmış oldukları çalışmada farklı oranlarındaki laurik asit/oleik asit karışımlarını kaplama materyali olarak kullanarak elde edilen C vitamini mikropartiküllerinin % 89'dan % 98'e değişen toplam enkapsülasyon etkinliğine sahip olduğunu, enkapsülasyon etkinliğinin ise taşıyıcıdaki laurik asit miktarı ile ters orantılı olduğunu gözlemlemişlerdir. Ayrıca taşıyıcı materyalde bulunan yüksek miktardaki oleik asit içeriğinin, enkapsülasyon etkinliğini yükseltmesinin yanı sıra elde edilen partikül çaplarının büyük olması ve yüksek oranda aglomerasyon gibi dezavantaj oluşturabilecek durumlar gözlenmiş, uygun miktar ve koşullar ile başarılı bir enkapsülasyonun sağlanabildiği rapor edilmiştir. Alvim vd. (2016) sprey soğutma ve sprey kurutma tekniği ile C vitamininin kapsüllenmesini incelediklerı bir diğer çalışmada; bisküvi üretimi esnasında kapsüllenmemiş C vitamini ile elde edilen üründeki içeriğe kıyasla, kapsüllenmiş C vitamini ile elde edilen ürünlerdeki içeriğin (pişirme esnasında) korunması sebebiyle avantajlı olduğu belirtilirken, kapsüllenmiş olan bu aktif maddenin (pişirme sırasında) termal bozunumuyla ilişkili olan bisküvi üzerinde koyu renkli lekelerin oluşması engellenmiştir. Schrooyen vd. (2001) de sprey soğutma tekniği ile elde ettikleri stabilitesi arttırılmış C vitamini mikrokapsüllerinin ekmek, bisküvi ve bazı tahıllara katkılamada avantajlı olduğunu raporlamışlardır. Matos-Jr vd. (2015) hidrojene palm yağı ve bitksel gliserol monostearat kaplama materyallerini kullanarak, farklı askorbik asit miktarında sprey soğutma yöntemi ile elde edilen mikroparçacıklarının; verim, morfoloji, ortalama boyut ve dağılımı, kapsülleme verimliliği ve askorbik asit kararlılığı açısından karakterize edilmesi üzerine çalışma yürütmüşlerdir. Sonuçlar lipit türüne bakılmaksızın, partikül büyüklüğünün askorbik asit konsantrasyonu ile ilgili olduğunu ortaya koymuştur. Yani aktif madde konsantrasyonu ne kadar düşük ise parçacık boyutu da o kadar küçüktür. Parçacık boyutunun küçük olması yüzeysel alanını arttıracağından, partikül yüzeyinde aktif malzeme bulma ihtimali de artacaktır. Ayrıca düşük aktif bileşen konsantrasyonunun gözlemlenen kapsülleme verimini de bir o kadar düşürdüğü anlaşılmıştır. Matos-Jr vd. (2017) askorbik asidin sprey soğutma yöntemi ile kapsüllenmesinde kaplayıcı materyal olarak palm ve palm çekirdeği yağı; emülsifiyer olarak soya lesitini ile farklı besleme formları ve karıştırma hızlarında denemeler gerçekleştirmişlerdir. Bu çalışmada önerilen iki besleme hazırlama yöntemi arasındaki kıyaslama sonucunda, her iki yöntemin de avantaj ve dezavantajları olduğunu; C vitaminin stabilitesi ile ilişkili olarak daha umut verici sonuçlar elde edildiği raporlanmıştır. Paucar vd. (2016), sprey soğutma ile D_3 vitamininin enkapsülasyonu üzerine yaptıkları çalışmada taşıyıcı matris olarak bitkisel yağ, soya lesitini ve balmumu kullanılmışlardır. D_3 vita-

mini yüklü partiküllerde (oda sıcaklığında, 65 gün) depolama sonrası ölçümü ile saptanan aktif bileşiğin, immobilize olmayan vitamin grubuna kıyasla korunduğu ve diğer tüm formülasyonlar içerisinde balmumu eklenerek elde edilen formülasyonun D_3 vitamini stabilitesini geliştirdiği görülmüştür. Zoet vd. (2011), D vitamininin kapsülasyonu üzerine yaptıkları çalışmada taşıyıcı matris olarak hidrojene palm yağı, pamuk yağı, susam yağı, carnauba mumu ve balmumu kullanmışlardır. Erime noktası 45-90 °C aralığında değişebilen taşıyıcı matrislerin, mikrokapsül kalitesini etkilediği ve elde edilen D vitamini mikrokapsüllerinin gıda veya yem katkısı olarak kullanılabileceği bildirilmiştir. Sprey soğutma tekniği ile E vitaminini (α-tokoferol) kapsülleyen Gamboa vd. (2011) hidrojene soya yağı ve normal soya yağını kaplama materyali olarak kullanmışlardır. Çalışma sonunda E vitaminin mikroenkapsülasyonunun %90'lara varan oranlarda verime sahip olduğu ve E vitamini kapsüllerin depolama süresince (180 gün) iyi bir stabilite de seyrettiği rapor edilmiştir.

Tablo 1. Sprey Soğutma Yöntemi ile enkapsüle edilmiş gıda uygulamaları

Table 1. Encapsulated food applications with spray cooling method

Çekirdek Materyal	Kaplama Materyali	Enkapsülasyon İşlem Koşulları	Kaynak
Zencefil Oleoresin	Palmitik Asit (Erime noktası: 63°C) Oleik asit (Erime noktası 8.2 ±0.1°C) Palm yağı (Erime noktası: 44.9 ±0.2°C)	Emülsiyon hazırlama/ homojenizasyon: Kaplama materyalleri 80°C'ye ısıtılır. Atomizasyon: 0.7 kg/st besleme hızı 7 °C hava giriş sıcaklığı 1052 L /s atomize hava hızı 35.000 L/s soğutma havası akış hızı	**Oriani vd. (2016)**
Gallik Asit	Hidrojene Soya Yağı / Soya Yağı (Erime noktaları:71,30 -74,99°C) PGPR (Poligliserol polirisinoleat)	Emülsiyon hazırlama/homojenizasyon: Kaplama materyalleri 80°C'ye ısıtılır. Atomizasyon: 0.530 L /s besleme akış hızı 35,000 L /s soğutma havası akış hızı 667 L/s atomize edici hava akış hızı Giriş ve çıkış soğutma havası sıcaklıkları: 7 ve 11 °C	**Consoli vd. (2016)**
A vitamini, İyot, Demir	%1 Soya Lesitini Hidrojenize Palm Yağı (Erime noktası:63°C)	Emülsiyon hazırlama/homojenizasyon: Kaplama materyalleri 80-90°C'ye ısıtılır. Atomizasyon: Soğutma sıvısı olarak nitrojen kullanılmıştır.	**Wegmuller vd. (2006)**
Askorbik Asit	Laurik Asit/ Oleik Asit (Erime noktaları 41.6-48.4°C) PGPR 90 (Poligliserol polirisinoleat)	Emülsiyon hazırlama/homojenizasyon: 30000 rpm'de 5 dakika $5.28.10^4$ m^3/st besleme akış hızı 0.66 m^3/s atomize edici hava hızı 35 m^3/s soğutma havası akış hızı Giriş ve çıkış soğutma havası sıcaklıkları:6 ve 9.5°C	**Sartori vd. (2015)**
C vitamini	Sprey soğutucu için: Stearik Asit (Erime noktası: 55°C) Hidrojene bitkisel yağ (Erime noktası: 41°C) Sprey kurutucu için: Gam arabik	Sprey Kurutucu / Sprey Soğutucu Örnek sıcaklığı: 40 ±2 / Örnek sıcaklığı:70 ±2 Giriş sıcaklığı 150 ±2 / Giriş sıcaklığı 5 ±3 Çıkış sıcaklığı 75 ±3 / Çıkış sıcaklığı 15 ±2 Besleme hızı 8mL/dak / Besleme hızı 12 mL/dak	**Alvim vd. (2016)**
C vitamini	Hidrojene palm yağı (Erime noktası: 74.02°C) Bitkisel gliserol monostearat (Erime noktası: 89.51°C)	Emülsiyon hazırlama/homojenizasyon: Taşıyıcı matrisi oluşturan yağlar erime noktalarının 10°C üzerinde ısıtılır. Askorbik asit öğütülür (100µm'den küçük) Atomizasyon: 4 bar hava basıncı 22 ± 3°C soğutma odası sıcaklığı	**Matos-Jr vd. (2015)**
C vitamini	Palm ve palm çekirdeği yağı (Erime noktası: 43°C) Soya lesitini	Emülsiyon hazırlama/homojenizasyon: Taşıyıcı matrisi oluşturan yağlar erime noktalarının 15°C üzerinde ısıtılır. 523 rad/s homojenizasyon Atomizasyon: Besleme akışı 50 mL/dak 13 ±2 soğutma odası sıcaklığı 216 kPa atomizör basıncı	**Matos-Jr vd. (2017)**

D_3 vitamini	Bitkisel yağ (Erime noktası: 49°C) Soya lesitini Bal mumu	Emülsiyon hazırlama/homojenizasyon: Taşıyıcı matrisi oluşturan yağlar 80°C'ye ısıtılır. 5000 rpm'de 1 dakika Atomizasyon: 50 mL/dak besleme akış hızı 2.2 kgf/cm^2 hava basıncı 13 ±1ºC soğutma havası giriş sıcaklığı	**Paucar vd. (2016)**
E vitamini	Hidrojene soya yağı / hidrojene palm yağı (Erime noktası: 61°C)	Emülsiyon/Homojenizasyon: 5 dakika boyunca homojenizasyon	**Gamboa vd. (2011)**
% 10 likopen içeren ayçiçeği yağı	Hidrojene ve interesterifiye pamuk tohumu, soya ve palmiye yağlarından oluşan shortening (Erime noktası: 51°C)	Homojenizasyon/Besleme öncesi: Likopen daha önce 60 °C'de eritilen shortening Atomizasyon: 1kgf / cm^2 hava basıncı 40 mL/dak besleme akışı 13°C soğutma odası	**Pelissari vd. (2016)**
Lactobacillus acidophilus (LA), Bifidobacterium animalis subsp. laktis(BL)	Bitkisel yağ (Erime noktası:51°C)	Homojenizasyon: 7000 rpm'de 60 s Besleme öncesi: Dispersiyon 51°C su banyosunda manyetik karıştırıcı ile karıştırılır. Atomizasyon: 15 ±2°C soğutulmuş oda 5 bar hava basıncı	**Bambi vd. (2016)**
Lactobacillus acidophilus	Palm ve Palm çekirdeği yağı (Erime noktası: 43.34°C) Prebiyotikler İnulin ve polidekstroz	Homojenizasyon: 7000 rpm'de 60 s Atomizasyon: 15 ± 2°C soğutulmuş oda sıcaklığı 5 bar hava basıncı	**Okuro vd. (2013)**
Fitosterol	Stearik asit Hidrojenlenmiş bitkisel yağ (Karışım erime noktaları: 44.5-53.4°C)	Emülsiyon hazırlama/ homojenizasyon: Kaplama materyalleri 70°C'ye ısıtılır. Atomizasyon: Hava akış hızı 500-600 NL / st Giriş sıcaklığı 5 ± 2 °C	**Alvim vd. (2013)**
Glikoz çözeltisi	Stearik asit Oleik asit Yağ karışımlarının erime noktaları: 49.6-56.6°C Lesitin	Emülsiyon hazırlama/ homojenizasyon: Kaplama materyalleri 70°C'ye ısıtılır. 10000 rpm'de 5 dak Atomizasyon: 1.25 kgf / cm^2 hava basıncı 0°C' soğutulmuş oda sıcaklığı Her bir lipid karışımı oranları için tanka eklenmek için ideal sıcaklık ve atomizer sıcaklığı 60-65°C arasında değişir.	**Ribeiro vd. (2012)**
Soya Proteini Hidrolizatı	Hidrojene pamuk tohumu yağı (Erime noktası: 51°C) Poligliserol poliasinoleat (PGPR, %1) Tween 80 (%1) Soya lesitini (%2, %5, %7)	Emülsiyon hazırlama/homojenizasyon: Yağ karışımları 3600 rpm'de 30 s Soya Proteini Hidrolizatı:yağ (1:10 , 1:5) 6000, 8000 ve 10,000 rpm 1, 5 ve 7 dak. Atomizasyon: 45 mL/dk besleme akışı 15 ±2°C'de soğutulmuş odaya 2.2 kgf/cm^2 basınç	**Salvim vd. (2015)**

İyot, demir ve A vitamininin stabilitesini artırmak için kararlı bir tuz geliştirmek amacıyla Wegmuller vd. (2006) yaptıkları çalışmada hidrojene palm yağını taşıyıcı olarak kullanmışlar ve sprey soğutma ile potasyum iyodat, ferrik pirofosfat ve retinil palmitatı mikroenkapsüle etmişlerdir. Sprey soğutma ile elde edilen mikrokapsüllerin boyut ve morfolojisi ile iyot ve A vitamini kaybı incelenmiştir. Çalışmada elde edilen mikrokapsüller yerel bir tuz içerisine eklenip; 6 ay süre ile depolanmıştır. Depolama süresince tuzda meydana gelen renk değişiminin kabul edilebilir düzeyde ve retinil palmitatın stabilitesinin yüksek olduğu bulgulanmıştır. Ayrıca mikroenkapsül içeren ve mikroenkapsül içermeyen tuz ile gerçekleştirilen duyusal analiz sonuçları arasında fark bulgulanmamıştır. Sonuçlar sprey soğutma ile mikroenkapsüle edilmiş tuzlarla katkılamanın iyot, demir ve A vitamini depolama stabilitesini artırdığını göstermiştir.

Consoli vd. (2016) hidrojene soya yağı ve normal soya yağı karışımını, taşıyıcı matris olarak kullanarak sprey soğutma tekniği ile gallik asidi kapsüllemişler ve yağ karışımlarını oluşturan bileşimlerinin parçacık boyutunu etkilediği, yüksek konsantrasyonlardaki hidrojene soya yağına sahip formülasyonlardan elde edilen parçacıkların, boyutlarında azalmanın gözlendiğini bildirmişlerdir. Çalışma sonunda kaplama materyali içerisinde bulunan yüksek konsantrasyonlardaki hidrojene soya yağının kapsülleme verimliliğini arttırdığı, en iyi formülasyonlarda ise Gallik asit mikroenkapsülasyonunun %80 ve üzeri oranlarda verime sahip olduğu rapor edilmiştir. Yağ asitleri ile yapılmış bir çalışmada ise sprey soğutma teknolojisi ile elde edilen stearik asit mikropartiküllerine üretim esnasında oleik asit eklemesinin etkisi incelenmiştir. Oleik asit eklemesinin lipit kristali oluşumunu olumlu yönde etkilediği, sprey soğutmada yüksek verim elde edildiği, yağ taşıyıcı fazı ile enkapsülasyon uygulamasının oldukça avantajlı bir uygulama olduğunu bildirilmiştir (Ribeiro vd., 2012). Alvim vd. (2013) stearik asit ve hidrojene bitkisel yağ karışımlarının kaplama materyali olarak kullanıldığı çalışmalarında fitosterolü kapsülleyerek, fitosterol gibi hidrofobik bileşiklerin sprey soğutma yöntemi ile enkapsülasyon verimi ve aglemerasyon bakımından başarılı bir kaplama gerçekleştirilebileceğini bildirmişlerdir.

Soya protein hidrolizatının sprey soğutma ile mikronekapsülasyonu amacıyla yapılan bir çalışmada, yapılan bu işlemin soya protein hidrolizatının istenmeyen tadını maskelediği, daha dayanıklı bir forma getirildiği ve yağ sindirimi sırasında bağırsakta salınımını geliştirdiği bulgulanmıştır (Salvim vd., 2015).

Pelissari vd. (2016) likopenin kapsüllenmesi üzerine yapmış oldukları çalışmada çeşitli kaplama materyalleri ile parçacıkların yapısal özellikleri ve likopen kararlığını incelemişler; likopenin korunması için en iyi koşulların taşıyıcı olarak gam arabik kullanıldığı, sıcaklığın ve vakumun düşük olduğu yerlerde depolanması gerektiği rapor edilmiştir. Ayrıca buna ek olarak, taşıyıcı kompozisyona gam arabik ve karboksimetil selüloz ilavesinin parçacıkların morfolojisini etkilemediği gözlenmiştir.

Probiyotik Uygulamaları

Probiyotikler dahil çeşitli bakteri kültürlerinin enkapsülasyonu, genel enkapsülasyon faydalarının yanı sıra; gıda üretim proseslerinde, depolamada ve gastrointestinal sistemden geçerken canlılıklarının korunması bakımından avantaj sağlamakta olup, çeşitli tekniklerin yanı sıra sprey soğutma yöntemi ile de gerçekleştirilebilmektedir (Ünal ve Erginkaya, 2010).

Bir probiyotik olan *Lactobacillus acidophilus*'un, sprey soğutma teknolojisini kullanılarak; palm-palm çekirdeği yağı ile kapsüllenmesi ve bu kapsüllerin değerlendirmesini yapan Okuro vd. (2013), sprey soğutma işleminin canlılığı azaltmadığını ve mikroenkapsüle edilmiş probiyotiklerin enkapsüle edilmeyen probiyotiklere kıyasla daha stabil olduğunu; ayrıca katı lipid mikropartiküllerinin *Lactobacillus acidophilus* hücrelerini mide ve bağırsak sıvılarının etkilerinden koruyabildiğini bulgulamışlardır. Probiyotiklerle yapılan bir diğer çalışmada *Bifidobacterium lactis* ve *Lactobacillus acidophilus*' un mikroenkapsülasyon ile korunmasını amaçlayan de Lara Pedroso vd. (2012), sprey soğutma yöntemi ile enkapsüle edilmiş olan toz ürünlerin, simüle edilmiş barsak ve mide sıvılarındaki stabiliteleri ve 90 günlük depolama süreleri boyunca stabilitelerini incelemişlerdir. Sonuç olarak palm ve palm çekirdeği yağını kullanarak elde edilen probiyotikli mikrokapsüllerin mide ve bağırsak sıvılarından geçmesine karşı etkili olduğu ve düşük sıcaklıklarda depolanabildiği gözlenmiş olup, sprey soğutma yönteminin oldukça avantajlı ve kullanılabilir bir yöntem olduğu rapor edilmiştir. Bampi vd. (2016) *Lactobacillus acidophilus* ve *Bifidobacterium animalis subsp. laktis*'in sprey soğutma tekniği kullanarak mikrokapsülasyonu sonrası canlılıklarını değerlendirmişler ve bunları tuzlu tahıl barlarına eklemişlerdir. 120 günlük depolama süresi boyunca barlardaki canlı hücre sayısı, tuzlu tahıl barlarına aktifleştirilmiş ve liyofilize edilmiş probiyotikler eklemek için kullanılan diğer yöntemlere kıyasla mikrokapsüllenmiş yapıların canlılıklarının fazla olmasından dolayı avantaj sağladığı raporlanmıştır.

Sprey soğutma yöntemi kullanılarak gerçekleştirilen enkapsülasyon çalışmalarından görüldüğü üzere aktif maddenin tipine bağlı olarak kullanılacak olan kaplama materyal(ler)inin tipi ve/veya oranı değişim göstermektedir. Ayrıca çalışmalarda araştırmacılar tek bir kaplama materyali kullanmak yerine iki veya daha fazla farklı kaplama materyalinin bir arada kullanmışlardır. Sprey soğutma yöntemi ile enkapsülasyon işleminde kaplama materyal(ler)inin tipi veya oranın önemli olduğu kadar dispersiyon hazırlama aşamasında uygulanan homejenizasyon hızının ve katılaştırma çemberinin sıcaklığı da oldukça önemlidir.

Sonuç

Sprey soğutma yöntemi ile sıvı/yarı sıvı ya da akışkan hale getirilebilecek hassas gıda bileşenleri dispersiyon haline getirilir ve atomizör sistemi aracılığıyla küçük damlacık boyutuna küçültülerek enkapsüle edilir. Yapılan çalışmalar incelendiğinde kaplama materyali olarak kullanılan mumlar, hidrojenize yağlar, lipit türevleri, jel oluşturma özelliği iyi olan protein ve karbonhidratların tek başına kullanılabildiği gibi, farklı kombinasyonlarda kullanılmaları durumda beklenen stabilite ve etkinliği geliştirerek başarılı bir enkapsülasyon sağladıkları bilgisine ulaşılabilmektedir. Ayrıca kaplanmak istenen çekirdek materyalinin istenmeyen tat, koku ve aktivitesinin maskelenmesi gibi faydaları yanı sıra düşük sıcaklıklarda gerçekleşmesi bakımından çoğu enkapsülasyon yönteminden daha fazla avantaj sağlamakta olan sprey soğutma yöntemi; diğer enkapsülasyon yöntemlerine kıyasla düşük işleme maliyetli ve kolay ölçeklendirilebilir yapısıyla öne çıkmakta olup, gelecek vadeden bir yöntem olduğu söylenebilinir. Fakat elde edilen son ürünün depolama ve kullanım süresince kapsül yapısının kırılıp-bozulması ile kontrolsüz salınımının gerçekleşmesi hedeflenenin aksine bir dezavantaja sebep olacağı ihtimalini de göz önünde bulundurmak gerektirdiğinden; kullanılacak kaplama materyali ya da materyallerinin elde edilen verilere dikkate alınarak seçiminin yapılması ve son ürün için uygun depolama koşullarının belirlenmesi gerekmektedir.

Kaynaklar

Alvim, I.D., Souza, F.D.S.D., Koury, I.P., Jurt, T., Dantas, F.B.H. (2013). Use of the spray chilling method to deliver hydrophobic components: physical characterization of microparticles. *Ciencia e Tecnologia de Alimentos*, 33(1), 34-39.

Alvim, I.D., Stein, M.A., Koury, I.P., Balardin, F., Dantas, H., Cruz, C.V. (2016). Comparison between the spray drying and spray chilling microparticles contain ascorbic acid in a baked product application. *LWT - Food Science and Technology*, 65, 689-694.

Bampi, G.B., Backes, G.T., Cansian, R.L., Matos-Jr, F.E., Ansolin, I.M.A., Poleto, B.C., Corezzolla, L.R., Favaro-Trindade, C.S. (2016). Spray chilling microencapsulation of *Lactobacillus acidophilus* and *Bifidobacterium animalis subsp. lactis* and its use in the preparation of savory probiotic cereal bars. *Food and Bioprocess Technology*, 9, 1422-1428.

Can Karaca, A., Low, N., Nickerson, M. (2013). Encapsulation of flaxseed oil using a benchtop spray dryer for legume protein-maltodextrin microcapsule preparation. *Journal of agricultural and food chemistry*, 61(21), 5148-5155.

Consoli, L., Grimaldi, R., Sartori, T., Menegalli, F.C., Hubinger, M.D. (2016). Gallic acid microparticles produced by spray chilling technique: Production and characterization. *LWT - Food Science and Technology*, 65, 79-87.

de Lara Pedroso, D., Thomazini, M., Heinemann, R.J.B., Favaro-Trindade, C.S. (2012). Protection of Bifidobacterium lactis and Lactobacillus acidophilus by microencapsulation using spray-chilling. *International Dairy Journal*, 26(2), 127-132.

Desai, K.G.H., Park H.J. (2005). Recent Developments in Microencapsulation of Food Ingredients. Drying Technology, 23, 1361-1394.

Gamboa, O.D., Gonçalves, L.G., Grosso, F.C. (2011). Microencapsulation of tocopherols in lipid matrix by spray chilling method. *Procedia Food Science*, 1, 1732-1739.

Garti, N., McClements, J.D. (2012), *Encapsulation technologies and delivery systems for food ingredients and nutraceuticals*. Woodhead Publishing, p. 110-130, ISBN 9780857091246

Koç, M., Sakin, M., Ertekin, F. (2010). Mikroenkapsülasyon ve gıda teknolojisinde kullanımı. *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi*, 16, 77-86.

Kwak, H.S. (Ed.). (2014). *Nano-and microencapsulation for foods*. John Wiley & Sons, p. 1-42, 223-248, ISBN 9781118292334

Lakkis, J.M. (2016). *Encapsulation and controlled release technologies in food systems*. John Wiley & Sons, p.116-177, ISBN 9781118733523

Matos-Jr, F.E., Comunian, T.A., Thomazini, M., Favaro-Trindade, C.S. (2017). Effect of feed preparation on the properties and stability of ascorbic acid microparticles produced by spray chilling. *LWT-Food Science and Technology*, 75, 251-260.

Matos-Jr, F.E., Di Sabatino, M., Passerini, N., Favaro-Trindade, C.S., Albertini, B. (2015). Development and characterization of solid lipid microparticles loaded with ascorbic acid and produced by spray congealing. *Food Research International*, 67, 52-59.

Okuro, P.K., Thomazini, M., Balieiro, J.C., Liberal, R.D., Fávaro-Trindade, C.S. (2013). Co-encapsulation of Lactobacillus acidophilus with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. *Food Research International*, 53(1), 96-103.

Oriani, V.B., Alvim, I.D., Consoli, L., Molina, G., Pastore, G.M., Hubinger, M.D. (2016). Solid lipid microparticles produced by spray chilling technique to deliver ginger oleoresin: Structure and compound retention. *Food Research International*, 80, 41-49.

Paucar, O.C., Tulini, F.L., Thomazini, M., Balieiro, J.C.C., Pallone, E.M.J.A., Favaro-Trindade, C.S. (2016). Production by spray chilling and characterization of solid lipid microparticles loaded with vitamin D 3. *Food and Bioproducts Processing*, 100, 344-350.

Pelissari, J.R., Souza, V.B., Pigoso, A.A., Tulini, F.L., Thomazini, M., Rodrigues, C.E. C., Urbano, A., Favaro-Trindade, C.S. (2016). Production of solid lipid microparticles loaded with lycopene by spray chilling: Structural characteristics of particles and lycopene stability. *Food and Bioproducts Processing*, 98, 86-94.

Ribeiro, M.M.M., Arellano, D.B., Grosso, C.R.F. (2012). The effect of adding oleic acid in the production of stearic acid lipid microparticles with a hydrophilic core by a spray-cooling process. *Food Research International*, 47(1), 38-44.

Rokka, S., Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology*, 231(1), 1-12.

Sagis, L.M. (2015). Microencapsulation and microspheres for food applications. Academic Press, p.235-248, ISBN 9780128003503

Salvim, M.O., Thomazini, M., Pelaquim, F.P., Urbano, A., Moraes, I.C., Favaro-Trindade, C.S. (2015). Production and structural characterization of solid lipid microparticles loaded with soybean protein hydrolysate. *Food research international*, 76, 689-696.

Sartori, T., Consoli, L., Dupas Hubinger, M., Cecilia Menegalli, F. (2015). Ascorbic acid microencapsulation by spray chilling: Production and characterization. *LWT-Food Science and Technology*, 63, 353-360.

Schrooyen, P.M.M., van der Meer, R., de Kruif, C.G. (2001). Microencapsulation: its application in nutrition, *Proceedings of the Nutrition Society*, 60, 475-479.

Ünal, E., Erginkaya, Z. (2010). Probiyotik mikroorganizmaların mikroenkapsülasyonu. *Gıda Dergisi*, 35(4), 297-304.

Wegmüller, R., Zimmermann, M. B., Bühr, V. G., Windhab, E. J., Hurrell, R. F. (2006). Development, stability, and sensory testing of microcapsules containing iron, iodine, and vitamin A for use in food fortification, *Journal of food science*, 71(2), 181-187.

Yajima, T., Umeki, N., Itai, S. (1999). Optimum spray congealing conditions for masking the bitter taste of clarithromycin in wax matrix. *Chemical and Pharmaceutical Bulletin*, 47, 220-225.

Zoet, F. D., Grandia, J., Sibeijn, M. (2011). Encapsulated fat soluble vitamin, NL Patent, 050668

Zungur, A. (2013). Mikroenkapsülasyon işleminin ekstra sızma zeytinyağı tozunun depolanması sırasında oksidatif stabilite, sorpsiyon ve fiziksel kalite kriterleri üzerine etkisi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İzmir.

Zungur, A., Güngör, Ö., Koç, M., Kaymak Ertekin, F. (2013). Emülsiyonların özellikleri ve emülsifikasyon koşullarının aroma ve yağların mikroenkapsülasyonu üzerine etkisi. *Akademik Gıda*, 11, 116-124.