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EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Investigation of *Salmonella* spp. of Contamination, Infection Routes and Their Effects on Public Health in Chickhen Meat

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Abstract

Chicken is cheap, healthy and nutritious food. Chicken that having an important role in human nutrition is an important source of development of microorganisms and pathogenic microorganisms, due to the appropriate composition and environmental conditions among animal foods. Therefore *Salmonella* serotypes are the most important ones among the pathogens isolated from chicken. In our study, 40 raw chicken samples taken from food enterprises producing poultry meat in the Aegean Region were examined anaylses of *Salmonella* spp. by using the Vidas *Salmonella* (Biomeriux) kit procedure by the mini VIDAS instrument. *Salmonella* species of the production stages are transmitted to chicken meat in different ways and these microorganisms can pass to humans and cause important health problems with the consumption of these products by causing various infections or intoxications (poisoning) in person. In this respect, the complete fulfillment of all hygiene rules, from production to sale, of these products will also eliminate the risks that may arise from public health.

Keywords: Chicken, VIDAS, *Salmonella*, Public health

INTRODUCTION

Today, it that increase in consumption of chicken meat and its products is a known fact. Because chicken meat which has high protein and low fat content and exhibits an appropriate unsaturated fatty acid composition enhances nutritional value. Chicken meat is also easy to prepare and market as food and hence widely used especially in fast-food restaurants (Mead, 2000). Chicken which having an important place in human nutrition is an important source of degradation of microorganisms and pathogenic microorganisms due to the appropriate composition and environmental conditions among animal foods.

Therefore, removal of microbial contamination sources or minimization of microbial contamination are one of the important points to be considered when producing a quality and safe product in the food industry.

Among the pathogens isolated from chickens are the most important and most important ones are *Salmonella* serotypes (Jørgensen et al., 2002). *Salmonella* that cause diarrheal disease in humans is a group of bacteria. Generally, it spreads through food contaminated with animal. There are many different types of *Salmonella*.

The most common *Salmonella* serotype was *Salmonella enteritidis*, followed this *S. paratyphi* B and *S. typhimurium*. Today, *S. enteritidis* that among the main causes of foodborne infections in *Salmonella* is the most common *Salmonella* serotype present in Turkey (Jørgensen et al., 2002).

Salmonella serotypes have been shown to be susceptible to commonly used antibiotics, but they have been shown to develop resistance to nalidixic acid, ampicillin, tetracycline and streptomycin (Bailey et al., 2001). Poultry is the most important source of *Salmonella* in animals. Transmission of chicken meat which has a high risk for *Salmonella* is caused by the passage of *Salmonella* intestinal flora directly or indirectly into the operating environment and hence to the poultry carcasses during the removal of the intestines and deeply excised and disposable parts of the poultry slaughterhouses. Decontamination methods for the removal of microorganisms from chicken carcasses are generally applications with a limited effect and purpose of reducing the microbial population. The proportion of *Salmonella* that is transmitted to carcasses in cutting of chickens grown on farms where *Salmonella* has become a native flora is naturally high (Chang, 2002).

In our research, it was aimed to carry out *Salmonella* analysis in the samples obtained from companies that produce chicken meat and to investigate ways of transmission of these microorganisms to chicken meat and to examine these products in terms of public health.

MATERIAL and METHOD

In our study, 40 raw chicken samples were used as material from different white meat production and sales companies in the Aegean Region and analyzes of *Salmonella* spp were performed. Samples were taken into sterile sample bags as reported in ISO 18593 (2004), brought to the laboratory in the cold chain and analyzed in the same day. Samples were stored at +4° C until analysis resulted. *Salmonella* assays for chicken samples were performed by using the Biomerieux Vidas SLM protocol of AOAC OMA (2004). AOAC OMA (2004) of VIDAS *Salmonella* is an enzyme linked fluorescence immuno assay used tecnique of ELFA (enzyme linked fluorescence assay) for the detection of *Salmonella* antigens in VIDAS devices. According to the protocol, 25 g of food samples were aseptically transferred to 225 mL Buffered Peptone Water (Merck-107228), homogenized and incubated at 37±1° C for 16-22 hours. After the incubation, 0.1 mL was taken and incubated for 22-26 hours at 41.5±1° C, transferred to 10 mL SX2 Broth (Biomeriux-1170250). After the incubation, 500 µL of SX2 Broth was inoculated into the Vidas *Salmonella* stribe and placed in the device.

According to the protocole of AOAC OMA (2004), the results are automatically analyzed by the device when the test is completed. The fluorescence is measured twice in the reading bath of the reactive styrene for each tested sample. The first reading is the background reading of the substrate bath before the Solid Phase Sorter (SPR) enters the substrate. The second reading is taken after the incubation of the substrate with the remaining enzyme inside the SPR. The RFV value (Relative Fluorescence Value) is calculated by subtracting the empty arrow from the final result. The RFV values that obtained for each sample were evaluated by the device as in Table 1.

Table 1. Vidas *Salmonella* threshold value and evaluation

Test Value	Evalution
<0.23	Negative
≥0.23	Posivite

Results that test values lower than the threshold value show there is no *Salmonella* antigen in the sample or is below the detection limit of the concentration of *Salmonella* antigen. Samples that test values equal to or greater than the threshold value have been shown to be contaminated with *Salmonella* (6). In this case, the verification of the positive results was carried out according to the verification steps in (AOAC OMA, 2004).

RESULTS and DISCUSSION

Salmonella analysis results of raw chicken samples are shown in Table 2.

Table 2. The results of Vidas *Salmonella* analysis of chicken samples

Sample No	Result	Sample No	Result	Sample No	Result	Sample No	Result
1	<0.23	11	<0.23	21	<0.23	31	<0.23
2	<0.23	12	0.55	22	<0.23	32	<0.23
3	<0.23	13	<0.23	23	<0.23	33	<0.23
4	<0.23	14	<0.23	24	<0.23	34	2.78
5	<0.23	15	<0.23	25	<0.23	35	<0.23
6	1.36	16	<0.23	26	<0.23	36	<0.23
7	<0.23	17	2.55	27	0.83	37	<0.23
8	<0.23	18	<0.23	28	<0.23	38	<0.23
9	<0.23	19	<0.23	29	<0.23	39	<0.23
10	<0.23	20	3.41	30	<0.23	40	<0.23

Note: <0.23: *Salmonella* spp. was notdetected, ≥0.23: *Salmonella* spp. was detected

6 out of 40 raw chicken samples were tested *Salmonella* spp. as a result of analysis. According to the results in Table 2, 6 samples which are found to be *Salmonella* are not in compliance with the limits specified in Turkish Food Codex Microbiological Criteria Regulation (2011) (Table 3).

Table 3. *Salmonella* limit value according to the Turkish Food Codex Microbiological Criteria Regulation in raw poultry meat

Food	Microorganism	Limits
Raw poultry meat	*Salmonella*	0/25g-mL

Especially, the dangerous organisms, such as *Salmonella* cause serious health problems like bloody diarrhea, fever and abdominal cramps in humans. In our study, *Salmonella* spp. was detected in 6 out of the 40 raw chicken samples, species of *Salmonella* spp. contamine to chicken meat that affect consumers` health seriously.

Efe et al. (2005) performed a total of 50 chickens, skin and breast samples of 18%, 26% and 16% of *Salmonella* spp. in Ankara, in *Salmonella* studies on chicken meat. In addition, Sezen (2009) performed *Salmonella* spp. in 6 samples of analysis of 175 poultry meat samples in a study in Istanbul.

CONCLUSION

As a result, it that to reduce or prevent the food-borne pathogens or the microorganisms the controls to be made at critical control points in food enterprises is very important for the public health. Therefore, in order to reduce the risk of contamination in poultry cuttings should be taken into consideration to raise healthy and *Salmonella*-free poultry. Starving chicken before slaughter, cleaning and disinfection of tools and materials at every step of the cutting process, controlling constantly the temperature of the required areas, attention to personnel hygiene, observing slaughterhouse sanitation, establishing a laboratory for autocontrol in every slaughterhouse can prevent contamination of *Salmonella* species to chicken meat.In this way, this food contamination which constitutes a significant risk agent in terms of public health can be passed.

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EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Determination of Table Olive and Olive Oil Consumption and Preference of Children from Yalova/Turkey

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Abstract

This research aimed to determine consumption amount of table olive and choice of olive oil of children from elementary schools of Yalova/Turkey. For this aim 497 questionnaire forms were conducted with parents of children. Results of this study will provide some information about table olive and olive oil consumption of children. That will be useful for policy maker and producer which willing to increase their table olive and olive oil production in Turkey. In this study table olive and olive oil consumption of children were found at low level only 38 % of children consume table olive every day. 60 % of children did not consume olive out of breakfast so that bread, appetizer or bakery products produced with table olive can be use a toll to increase consumption of children. Only 39 % of children like to consume olive oil in foods and 68 % of children never asked to use olive oil in cooking. Sweeter and more delicate olive oils should be produced to increase consumption of children.

Keywords: consumption, olive oil, olive oil in cooking, prefer, table olive

INTRODUCTION

Turkey is an important olive producer but olive oil consumption is only one 1.9 l per person in a year (IOC, 2015b). According to 2015 years report of the International Olive Council, Turkey has soared table olive consumption from 110 000 t in 1990/91 to 350 000 t in 2014/15. Turkey has personnel table olive consumption between 4.0 and 4.7 kg per year (IOC, 2015a). The share of the table olive production of Turkey in the world is about 17 %. Turkey is the number one country in the world for producing black olives. Moreover, 11% of the world table olive consumption was provided by Turkey (Anonymous, 2013). However olive and table olive consumptions were not at a satisfied level because there are new olive plantation at huge areas in Turkey. The main reason of the low table olive consumption was thought as the increases in interest of the corn flakes and fast food consumption instead of breakfast (contain table olive, chess, butter, etc.) and home food (Smith, 2009; Vijayapushpam et al., 2003). This consumption behavior changing trend was more dramatic in children. Food consumption behavior of a person takes form during childhood. So that children should be trained and informed to form a healthy food selection behavior for their life (Dollman et al., 2005; Smith, 2009). For this reason, children should be informed about which food has which effect on their body (IOC, 2015b). Thus this research focused on children from elementary schools of Yalova City Center/Turkey to determine their table olive and olive oil consumption behavior.

MATERIAL and METHOD

The material of the study was face to face filled consumer survey by parents of children from 18 elementary schools of city center of Yalova/ Turkey. Questionnaire form was used only one child from each family. This study completed between 1 October 2013 – 24 December 2013. Target group of the study was these children whose ages between 6 and 12 years old to measure the table olive and olive oil consumption habit. Survey forms according to research objectives and contents were prepared. As a result of grading and sorting of survey 497 research has been recognized as the ultimate material.

RESULTS and DISCUSSION

Socioeconomic and demographic factors such as age, gender, education and income were reported as affected factors on table olive and olive oil preferences of consumers (Kailis and Harris, 2007; Tumer, 2013). Demographic characteristics of 497 participants of this study were given in Table 1.

Table 1. *Demographic characteristics of participants who responded to the survey*

Gender	**Number of children**	**%**
Female	236	47.58
Male	261	52.42
Age	**Number of children**	**%**
6-8	163	32.79
9-10	136	27.36
11-12	198	39.83
Family income per month (Turkish Lira)	**Number of family**	**%**
<1500	32	6.44
1500-3000	247	49.70
3000<	218	43.86
Education time of parent (year)	**Number of parent**	**%**
<8	130	26.16
8-12	194	39.03
12<	173	34.81

Table olive is one of the main component of breakfast culture besides cheese and bread, the olive consumption rate increase depending on the size and members of the household of Turkey (Tumer, 2013). Percentage distribution of responses to the question of "Does your child consume olives in breakfast?" was shown in Figure 1. Increases in interest of the corn flakes and fast food consumption instead of Mediterranean traditional breakfast may cause a decline of table olive, chess, butter, etc. (Ozdemir, 2016; Smith, 2009). In this study everyday consumption of table olive in breakfast was detected for only 38 % of children. This result indicate us a need to increase the table olive consumption with healthy breakfast nutrition habit of children.

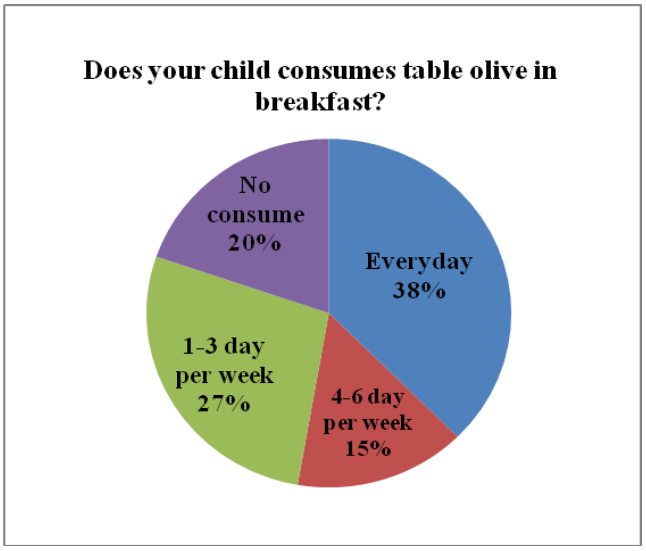

Figure 1. "Does your child consume olives in breakfast?" percentage distribution of responses to the question.

There has been found a positive relation between age of consumer and table olive consumption. The consumers between ages 26 and 45 consume more olives than the consumers under the age of 26 (Tumer, 2013). Percentage distribution of responses to the question of "Does your child consume olives except from breakfast?" was shown in Figure 2. National and international olive councils, olive producer associations support some promotion activities to increase table olive consumption. Increase of table olive consumption out of breakfast probably important factor for increase its total consumption. But result of this research showed that % 60 of children did not consume olive out of breakfast. Using table olives in production of bread, bakery products, appetizers or meals will help to increase its consumption in children.

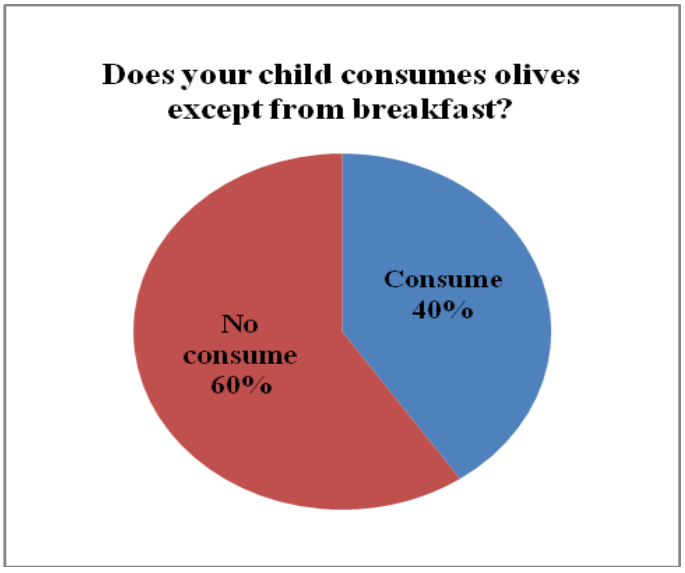

Figure 2. "Does your child consumes olives except from breakfast?" percentage distribution of responses to the question.

Nowadays, people, who want to live a long and healthy life, have understood the importance of this diet and they have begun consuming more olives, which is one of the most important elements belonging to Mediterranean diet. Percentage distribution of responses to the question of "How many olives does your child consume for a day?" was shown in Figure

3. Per capita olive consumption is 0.35 kg in Argentina, 1.88 kg in Greece, 3.13 kg in Egypt, 3.69 kg in Turkey and 3.88 kg in Spain (Anonymous, 2011). Turkey takes place in the fourth in the world olive consumption is because olives constitute a major part of traditional Turkish breakfast combined with bread, cheese, and black tea (Tumer, 2013). But this study showed that 20 % and 27 % of children no consume olive and consume only 1-3 olive per a day respectively. So that total 47 % of children had potential or increase their consumption of table olive.

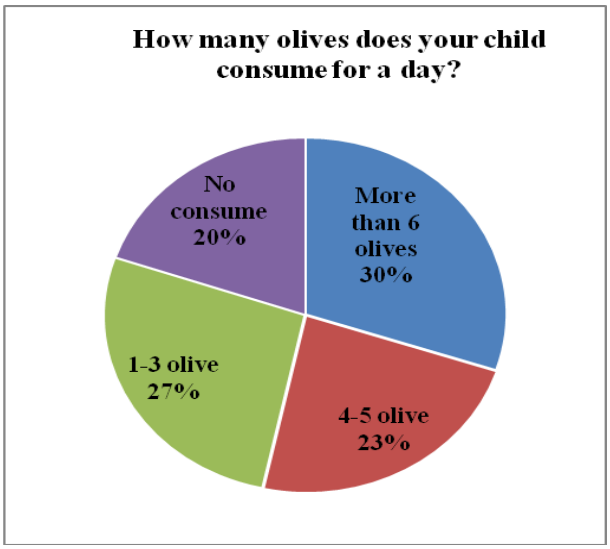

Figure 3. "How many olives does your child consume for a day?" percentage distribution of responses to the question.

High amount of table olive consumption among women should be gained the consciousness for the healing and other positive effects of the olive (against cancer and other coronary heart diseases) on the human health system (Tumer, 2013). Percentage distribution of responses to the question of "Does your child like to eat table olive?" was shown in Figure 4. Because of olive consumption, mothers will also lead and influence their children positively towards to the olive consumption. Thus, there will be a wide and conscious wave on the olive consumption not only for present, but also for the future generations (Tumer, 2013). But according to result of this study, 18 % and 22 % of children were not like and liked a little to consume olive respectively. This result may be solved by production of table olive to meet the sensory expectation of children. In general table olives had high salt content (5.3 - 6.75 %) and total titratable acidity (0.34 - 1.07 % lactic acid) so that children did not attracted by taste of table olives (Kailis and Harris, 2007). To increase attraction of children for table olive, new table olive production method and formulations which include lesser amount of salt and acidity should be developed.

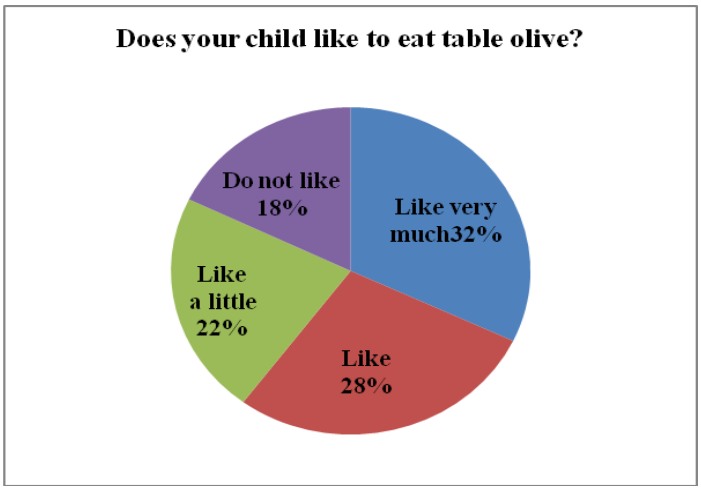

Figure 4. "Does your child like to eat table olive?" percentage distribution of responses to the question.

Dietary recommendations for children with hypercholesterolemia are aimed to reduce the saturated fat consumption and increase the intake of monounsaturated (MUFAs) and polyunsaturated fats (PUFAs). Mediterranean diet, which includes many vegetables, fruit, legumes, nuts, cereals and fish, in which fat represents about 35 % of the energy and comes mainly in form of MUFAs, especially from olive oil (Estevez-Gonzalez, 2010). In this research percentage distribution of responses to the question of "Does your child like to eat food with olive oil?" was shown in Figure 5. Olive oil is an example of a product for which consumption is marked by local culture; it is emblematic of the diet and culture of the Mediterranean region (Achabou et al., 2010). The consumption of skim milk enriched with olive oil increases the HDL cholesterol and apolipoprotein A-I levels in children with hypercholesterolemia (Estevez-Gonzalez, 2010). But in this research ratio of children like to consume olive oil in foods were detected at low levels (39 %). So that olive oil consumption of children should be increased by informing activates which designed according to children ages.

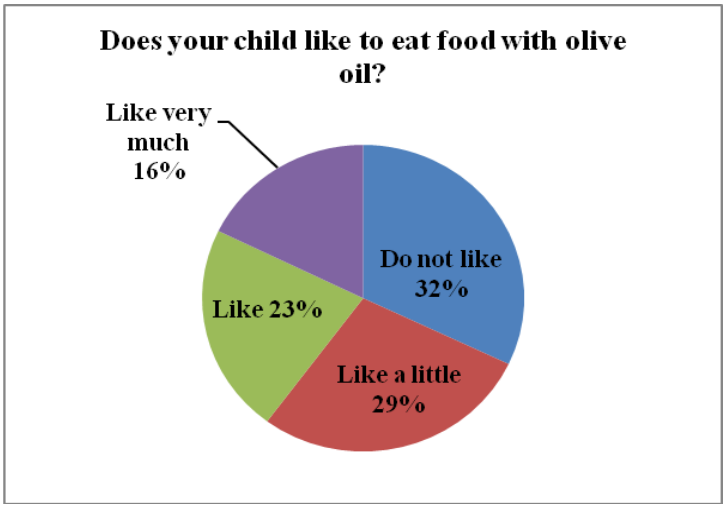

Figure 5. "Does your child like to eat food with olive oil?" percentage distribution of responses to the question.

Age was reported as a statistically significant factor and plays an important role on olive oil consumption choices among Turkish households (Yildiz Tiryaki, 2008). Percentage distribution of responses to the question of "Does your child ever asked you to use olive oil in cooking?" was shown in Figure 6. Olive oil consumption reported as reduce the total

cholesterol levels (7.2 %) and LDL-C (9.5 %), but there were no changes in HDL-C in children (Estevez-Gonzalez, 2010). Olive oil also stimulates bone growth and mineralization, and thus is recommendable both for children and the elderly (Arcas et al., 2013).

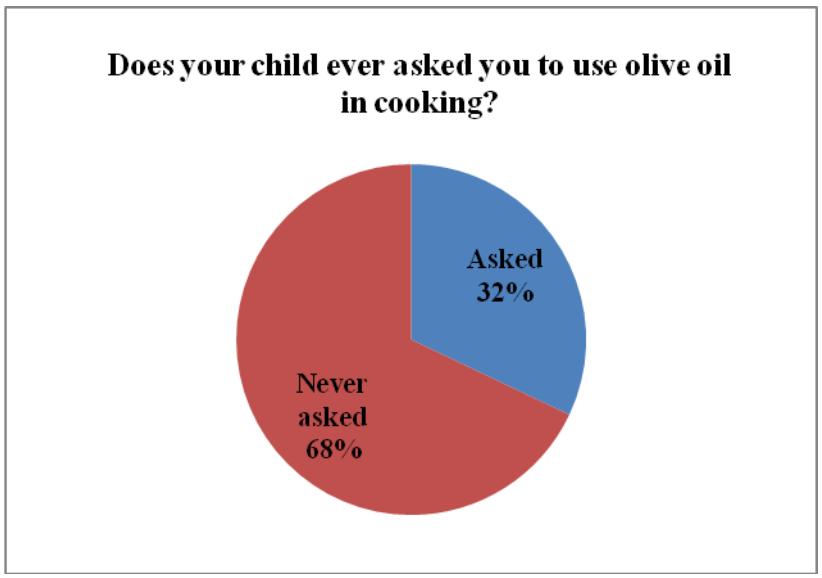

Figure 6. "Does your child ever asked you to use olive oil in cooking?" percentage distribution of responses to the question.

According to results % 68 of children never asked to parents to use olive oil in cooking. Thus there was need to developing studies on the taste of olive oil. By this sensory expectation of children will be met by producers. Similar result also discussed by Arcas et al. Sweeter and more delicate olive oils can be more consumed by children, who will not perceive any strong or unpleasant taste of olive oil (Arcas et al., 2013). Specially formulated olive oils which had smooth, sweet and fruity taste were also produced for children by some companies (Anon., 2013; Anon., 2016a). Increase in olive oil production with respect to sensory expectation of children will help to increase its consumption by children.

CONCLUSION

The objective of this study is to analyze the table olive and olive oil consumption of children in Yalova. A questionnaire has been conducted with the 497 parents of these children by using the face to face interview method in 2013. Parents answered the question about according to their observation in daily life of their children. According to the evaluation results of questionnaire forms; both table olive and olive oil consumption of children were at low levels 47 % and 39 %.

So that producer and marketing companies had potential to increase table olive and olive oil consumption of children. Future studies will be need to determine sensory expectation of children from table olive and olive oil.

By this way specific product can be developed for children to satisfy children expectation. Food consumption habit takes form during childhood so that informing children about healthy nutrition has significant importance. Consumption of olive oil and table olive; insures high health benefits and prevents from diseases especially cancer and cardiovascular diseases. Thus olive oil and table olive should be put in diet for a healthily life.

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Glycerol-Based Process Contaminants in Palm Oil

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Abstract

Palm oil is one of the common used vegetable oil in food industry throughout the the world. This tropical palm fruit is reddish in color because of rich beta-carotene content. There are two types of palm oils that can be produced from the palm fruit. First type is palm oil, and it is produced from the pulp of the palm fruit. Second type is palm kernel oil and it is produced from the kernel part of the fruit. Palm oil exposed to high temperatures may have changes in its lipid matrix which leads to the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters, additionally 2-monochloropropane-1,3-diol (2-MCPD) and glycidyl fatty acid esters. According to International Agency for Research on Cancer, 3-MCPD is a substances that have a possibility to cause cancer. European Food Safety Authority also has evaluated the risks of those substances for public health recently and stated that those substances are especially formed during the refining process of vegetable oils at high temperatures such as 200°C. There are two methods using for determining the esters of palm oil. First one is direct method based on LC-MS/MS which offers higher specificity and very low LOD/LOQ for each single 3-MCPD fatty acid esters, on the other hand it requires all set of standard materials for determination. The second method is indirect method including hydrolysis and derivatization of the analyte and determination step with GC-MS. In the scope of this study, 3- and 2- MCPD, their fatty acid esters, and glycidyl fatty acid esters occurrence during processes of palm oil, its regulation, its adverse effects on human health, and analytical methods for determining those type of esters are reviewed.

Keywords: Palm oil, Glycerol-based process contaminants, Analytical methods, Regulation, Health effects

INTRODUCTION

Palm fruit comprises a kernel enclosed in a shell called endocarp surrounded by pulp called mesocarp (Corley&Tinker, 2007). The color of the palm fruit is orange-red based on the higher content of the carotene. Palm oil is extracted from the ripened mesocarp of the fruits of palm oil tree (Elaeis guineensis). Crude palm oil and palm kernel oil are two different types of oils that can be obtained from the mesocarp and kernel of the palm, respectively. These oils are highly saturated in fatty acids. Their percentage is between 50% and 80% and they are esterified with glycerol. Palm oil exposed to high temperatures may have changes in its lipid matrix which leads to the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters, additionally 2-monochloropropane-1,3-diol (2-MCPD) and glycidyl fatty acid esters.

According to International Agency for Research on Cancer, 3-MCPD is a substances that have a possibility to cause cancer. European Food Safety Authority also has evaluated the

risks of those substances for public health recently and stated that those substances are especially formed during the refining process of vegetable oils at high temperatures such as 200°C. In the scope of this study, 3- and 2- MCPD, their fatty acid esters, and glycidyl fatty acid esters occurrence during processes of palm oil, its regulation, its adverse effects on human health, and analytical methods for determining those type of esters are reviewed.

Composition of Palm Oil

Palm oil and palm kernel oil includes higher amount of saturated fatty acids. Palm oil has semi solid state at ambient temperature and contains some various saturated and unsaturated fats in forms of palmitate (44%, saturated), oleate (39%, monounsaturated), linoleate (10% polyunsaturated), stearate (5% saturated), myristate (1%, saturated), linolenate (0.3% polyunsaturated) and glyceryl laurate (0.1% saturated) (Cottrell, 1991).

One tablespoon of processed palm oil is 120 calories and contains13.6 grams of fat, 2.17 mg of vitamin E and 1.1 mg vitamin K. Fresh red palm oil also contains higher amount of beta carotene.

Red colored palm oil is obtained in a process in which 80% of the original carotenoids level remain. Therefore, it can evaluated as natural source of carotenoids which enhances functionality of immune system and improves cardiovascular health. Carotenoids have antioxidant properties, therefore they preserve cells and tissues from the detrimental effect of free radicals.

Palm Oil Production: From Plantation To Final Use

After harvesting, the palm fruit is transported to the mills and sterilized with steam to inactivate enzymes and microorganisms (Gunstone, 2011). The fresh fruit bunches of the fruit needs to be processed within 48 hours. The palm fruit can produces two significant types of vegetable oils known as palm oil and palm-kernel oil. Another product is palm kernel cake which is used for livestock feed.

Processing and Refining

When the mesocarp is fully ripened palm fruits contains about 56–70% edible oil. This oil can be extracted by using different methods and these methods are classified by four pathways according to their degree of complexity. The basic operations used in the palm oil processing are fruit sterilization, fruit loosening/stripping, digestion, oil extraction and clarification.

Fruit sterilization denotes heat providing and moisture absorption. The goal of the process is to inactivate the lipolytic enzymes found in the mesocarp (Owolarafe et al., 2002; Poku, 2002).

There are two defined refining process for crude palm oil; physical refining and chemical refining. Physical refining includes degumming, bleaching, and deodorizing process where refined bleached deodorized palm oil (RBD) and palm fatty acid distillates are formed.

Two fractions can be obtained from RBD as palm stearin and palm olein. Chemical refining includes alkali neutralization, bleaching, deodorizing, neutralization and fractionation steps. At the end of the process palm stearin and palm olein are occurred (Ogan et al., 2015).

By-Products

Oil by-products including the empty fruit bunches, palm oil mill effluent, sterilizer condensate, palm fiber and palm shell are main byproducts that formed during the processing steps of palm. These by-products are used organic fertilizer in palm oil areas and also in the manufacture of soaps, animal feed while palm fiber and shell are used as fuel (Yusoff, 2006).

They are also widely used in the manufacturing of candles, cosmetics, toiletries and pharmaceutical products. Moreover, they are used in oleochemical industries and also used as a raw material in production of Vitamin E as well as production of animal feed (Tan, 2006).

Esters of Palm Oil

3-MCPD is known as a viscous liquid, it occurs as a racemic mixture of its enantiomers, (S)-(+) and (R)-(-) 3-MCPD (Velisek, 2002; Hamlet et al., 2004). Each enantiomer of 3-MCPD shows different toxicological properties. The (S)-enantiomer exhibits antifertility activity, while the supplementary (R)-enantiomer has a harmful effect on kidneys and 3- MCPD also showed genotoxicity in vitro (Lynch et al., 1998; Velisek et al., 2002).

3-Monochloropropane-1, 2-diol (3-MCPD) and other derivatived chloropropanols like 2-monochloro-propane-1,3-diol (2-MCPD) have been known as process contaminants occurred in thermally processed food products like bakery products, liquid seasoning as well as in refined oils. Besides, 3-MCPD is formed in the presence of sodium chloride that is naturally present in the food or added to the food (Hamlet et al., 2002; Hamlet et al., 2004; Franke et al., 2009; Baer et al., 2010). The amount of 3-MCPD increased at high temperatures between 100–230^{o}C and it could be reached its highest value at 230^{o}C. It is reported that the concentration 3-MCPD in unrefined oils and fats is in trace levels, and the main problem with 3-MCPD is related to the refining process for oils and fats (Pudel et al., 2011). Degumming with water, neutralization with potassium hydroxide and bleaching before deodorization can be rated among the useful techniques and methods to reduce and minimize the formation capacity of the palm oil in terms of 3-MCPD and glycidyl esters (Ramli et al., 2011). The lowest amount of 3-MCPD is generated in biscuits as they had a low salt content and their water content was below the optimum level (Calta et al., 2004).

For minimizing the 3-MCPD ester formation in edible fats and oils, acid dosage should be reduced based on the crude oil qualities. Otherwise acid degumming process should be substituted with other process. Besides, neutralization of the acidity prior to deodorization was shown, effective in reducing the formation of 3-MCPD esters (Weißhaar, 2008). In addition to processing conditions, growing conditions including regional, climatic, fruit cultivation, fertilization, and harvesting should be considered. In the future studies and researches, minimizing 3-MCPD esters will be influenced by the results of toxicological studies.

As a summary, in order to decrease esters and relevant compounds there exists three ways; removing of critical reactants from the raw material, changing of the refining process or removing of formed 3-MCPD esters and relevant compounds from the refined product (Henderson & Osborne, 2000).

Analytical Methods to Determine Esters of Palm Oil

To determine the esters of palm oil requires some sophisticated techniques including high pressure liquid chromatography (HPLC), gas chromatography equipped with mass spectrophotometric detector (GC-MS), and solid phase micro extraction (SPME). There are two methods using for determining the esters of palm oil. First one is direct method based on

LC-MS/MS which offers higher specificity and very low LOD/LOQ for each single 3-MCPD fatty acid esters, on the other hand it requires all set of standard materials for determination. The second method is indirect method including hydrolysis and derivatization of the analyte and determination step with GC-MS. Indirect method have an increased robustness, involve hydrolysis and derivatization. Also, it might induce chemical modifications of the analyte (Henderson&Osborne, 2000;Vicente et al., 2015). Recently, there is a promising new method has been released based on QuEChERS method (quick, easy, cheap, effective, rugged, and safe). The method includes direct analysis of the extract and it does not need derivatization and determination is based on gas chromatography–triple quadruple mass spectrometry (Genauldi et al., 2017).

Effects of Palm Oil and Their Esters on Health

Even though palm oil contains higher amount of saturated fatty acids, its preferential esterification at the sn-1 and sn-3 carbon of triglycerides decreases their absorption and metabolic effects. It is reported that there is no experimental data that enables to understand the relation between palm oil consumption and cancer, therefore it is reported if the limit set for saturated fatty acids intake is kept below %10 of total energy in the daily diet then adverse effects can be prevented (Marangoni et al., 2017). Regarding 3- MCPD, its occurrence in the consumed food attract attention of scientist due to its possible toxicological properties. According to International Agency for Research on Cancer, 3-MCPD is a substances that have a possibility to cause cancer. It is reported that the main target organ for 3-MCPD toxicity is the kidney, with chronic oral exposure resulting in nephropathy, tubular hyperplasia and adenomas (JECFA, 2002). On the other hand, there is a few data to determine the exposure of 3-MCPD to consumers, therefore it is recommended to get more analyses of 3- MCPD in foods (Boon and Biesebeek, 2016).

Regulations about Palm Oil and Their Esters

According to the Regulation (EU) No 1169/2011 on the provision of food information to consumers entered into application on 13 December 2014, specific information on the vegetable origin of refined oils and fats should be given on the food packaging. Therefore, food producers should indicate the specific type of vegetable fat and oil they used which includes palm oil. For free 3-MCPD in soy sauces and hydrolysed soy protein in the commission regulation (EC) 1881/2006 a limit of maximal 20 mg/kg exists and the Scientific Committee on Food (SCF) of the European Commission as well as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) defined a tolerable day intake (TDI) of 2 mg free 3-MCPD per kg body weight.

Provisions for methods of sampling and analysis for the official control of 3-MCPD are laid down in Commission Regulation (EC) No 333/2007.

CONCLUSION

Palm oil isn't only used in food sector, but also used in cosmetics, pharmaceutical products, among others. Palm oil exposed to high temperatures may have changes in its lipid matrix which leads to the formation of 3-monochloropropane-1,2-diol (3-MCPD) esters, additionally 2-monochloropropane-1,3-diol (2-MCPD) and glycidyl fatty acid esters. Eventhough, 3-MCPD is declared as a substances that have a possibility to cause cancer. On the other hand, there is a few data to determine the exposure of 3-MCPD to consumers, therefore it is recommended to get more analyses of 3- MCPD in foods. Formation of 3-MCPD in foods can be minimized if the process conditions are optimized and raw material with higher quality can be obtained.

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Chemical Migration from Plastic Types of Food Contact Materials

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Abstract

Foods are exposed to contact materials during all steps passed from farm to fork. Regulation (EC) No 1935/2004 was set in the European Union (EU) to provide safe FCMs and to explain the general requirements of the materials. Plastic materials and articles intended to come into contact with food are regulated by Commission Regulation (EU) No 10/2011. Annex I of Commission Regulation (EU) No 10/2011 contains the Union list of authorized monomers, additives, polymer production and other starting substances. There are 885 authorized food contact material substances in the list. These listed substances called as *"Intentionally Added Substances (IAS)"* can be used to manufacture plastic materials, with the restrictions and specifications established in the list. The contamination of foods due to the release of chemicals from packaging materials can be originated from the substances used in their formulation (IAS) but also from interactions between different ingredients, degradation products or from the presence of impurities in the raw materials (so called *"Non Intentionally Added Substances-NIAS").* The components from food contact materials must not migrate into the foods in unacceptable quantities. Therefore, substances used in the manufacture of FCMs are regulated with maximum limits that may migrate into foodstuffs without causing any health concerns. There are two migration limit set for plastic based materials and articles: *Overall Migration Limit* and *Specific Migration Limit* (SML). SML are set for individual authorized substances based on toxicological evaluation. In the scope of this study, plastic type of food contact materials are classified, and migration concept is explained, the regulations about FCMs and analysis method on chemicals migrated are reviewed.

Keywords: Food contact materials, intentionally added substances, non-intentionally added substances, migration limits, and plastic regulations

INTRODUCTION

Foods are exposed to contact materials including cutlery and dishes, containers, processing machine, and packaging materials during all steps passed from farm to fork. (Simeneau, 2008; EC, 2004). Food industry has been conducting research and development activities on food packaging to increase shelf life, keep the food quality at optimum level, attract consumer interests, and reduce waste. A package material for any type of food should minimize aroma and flavor losses, constitute an excellent barrier for gas and water, provide a perfect hermetically sealed seam, as well as have a good mechanical properties.

Food contact materials including food packaging are generally based on paper, metal, ceramic, aluminum, lacquers and coating, and plastic (Krochta, 2007; Driscoll & Rahman, 2007; Robert, 2012).

Food packaging is used to increase shelf life, to keep food quality at optimum level, to attract consumer interest, to facilitate the sale and distribution (Robert, 2012). Foods

packaging provides information to consumers on product name, brand name, net weight, manufacturer information, price, production date, as well as the nutrient values in addition to keeping food at the desired amount in a single vessel and making it easier to bring a number of units to be moved into a single cluster and use (Çinibulak, 2010). Therefore, food industry makes expenditures on the research and development activities of food packaging systems. The degree of the final product quality and safety, and consumer expectations from the ergonomic features of the package affects the acceptance criteria of a package material. A package material for any type of foods should minimize aroma and flavor losses, constitute an excellent barrier for gas and water, provide a perfect hermetically sealed seam, as well as have a good mechanical properties and offer chemical and biological protection against contamination (Simeneau, 2008). Glasses, metals, paper, ceramic, and plastics are the most used materials for food packages. Glasses are inert packaging material and its shows heat resistance to thermal processing has advantages of providing good strength under compression and heat. Glasses as well as metals like steel and aluminum act a barrier to gases, water vapor and aromas. Paper based packaging materials produced from wood pulp, rags, and other waste have been reported to be used since the seventeenth century. Ceramic type packaging materials including glass and pottery are produced at high temperatures from non-metal inorganic material produced by high temperatures (Krochta, 2007; Driscoll & Rahman, 2007). Plastic packaging materials are made up from polymers by adding additives, processing aids, catalysts, and plasticizers.

Chemical components of packaging materials may migrate in to foods when they contact with them. This type of transfer is called as chemical migration, which is a mass transfer operation. Diffusion the macroscopic movement of molecules from high to low concentration is the main mechanism in migration. The migrated chemicals from packaging materials can be originated from the substances used in their formulation and also from interactions between different ingredients, degradation products or from the presence of impurities in the raw materials. The duration of the contact between the material and food, temperature profile during interaction and the physicochemical behavior of the packaging material are the main drives for the migration (Simeneau, 2008).

Keeping consumer health safe, components of food contact materials shall not migrate into the foods. Therefore substances used in the manufacture of the packaging materials are regulated with maximum limits that may migrate into foodstuffs without causing any health concerns. To analyze migrated chemicals, food simulants are used to test migration in the scope of compliance with regulations. Sophisticated equipment such as liquid and gas chromatography equipped with mass spectrometry and inductively coupled plasma mass spectrometry have been used successfully for migration analysis so far.

In the scope of this study, plastic type of food contact materials, the chemical migrations from packaging materials in to food and simulants, the analytical techniques and the recent international legislations which regulate packaging materials and chemical migration are reviewed and discussed in details.

Plastic Type Food Contact Materials

The starting substances of a plastic materials are mainly monomers. They react with other starting substances to make a large chain structure called as polymer. Polymers constitutes the main structural component of the plastics (EC, 2011). That process is known as polymerization (Selke, 2005). By application of heat and pressure, those high molecular weight polymers can be molded to get required final products and shapes including films, trays, bottles, and jars (Krochta, 2007). Additive such as antioxidant, solvents including printing inks, plasticizers, thermal stabilizers, light stabilizers are benefited to formulate

plastic resin along with the base polymer (Driscoll & Rahman, 2007; Lau & Wong, 2000; Arvanitoyanni & Kotsanopoulos, 2014) . In addition, organic or inorganic materials can be used as printing agents to coat plastic materials and articles. Polymer additives improves plastic flexibility, and polymer resistance to degradation by heat and light (Krochta, 2007; EC, 2011).

Depending on the heat resistance, polymers can be grouped in to two main group as thermoplastic and thermoset polymers. Thermoplastic polymers are linear or branched without crosslinks between polymer chains and can be softened and molten when heated, and can return to their original condition once cooling is applied. Despite of thermoplastic polymer, thermoset polymers cannot easily molten, therefore it plays an important role for making package closures, especially in rigid closure. If a plastic compose of one type of monomer with a regular repeating unit in the structure, it is called homopolymer. On the other hand, if it is made from multi type of monomer with different molecular place in the structure, it is called copolymer. (Krochta, 2007; Selke, 2005).

Plastics materials used in production of food contact materials

The common polymers used in food packaging include high density polyethylene (HDPE) and low density polyethylene (LDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), ethylene vinyl alcohol (EVOH), polyvinylidene chloridecopolymers (PVDC), and ethylene vinyl acetate (EVA).

High density polyethylene (HDPE) is a linear polymer of ethylene, which can be represented as –(CH2–CH2)n–. Since HDPE has a pigmented structure, it makes the containers opaque. It is commonly used in plastic milk bottles. Its structure prevents degradation caused by light, and enables the product more desirable for marketing (Selke, 2005; Lee,et al., 2008).

Low Density Polyethylene (LDPE) is also polymer of ethylene, compared to HDPE it is polymerized at high temperature and pressure. Therefore its polymer structure has branched structure. LDPE can be used in bread bags and squeezable drink bottles. Moreover it can be used as a heat seal layer and a moisture barrier (Selke, 2005; Lee, et al., 2008).

Polypropylene (PP) has high crystallinity, very low density and good clearness. Its monomer is propylene. The advantages of PP compared to PE, it has higher tensile strength, stiffness, hardness and a higher melting temperature (Luciano & Sara, 2016).

Polyethylene Terephthalate (PET), thermoplastic material, includes polymerized units of the monomer ethylene terephthalate. It is mostly used in plastic bottles for carbonated soft drinks and drinking water etc. (Selke, 2005).

Polystyrene (PS) is an aromatic polymer made from styrene with a benzene ring attached to every carbon. The brittleness and low impact strength are the characteristic features of PS. Since its thermal conductivity is low, it can be used insulating material. It is mostly used in disposable cups for hot beverages (Selke, 2005; Robert, 2012).

Polycarbonate (PC) is synthesized from its monomer Bisphenol A. Since its strength is high, it is successfully used for refillable water and milk bottles (Selke, 2005).

Polyvinyl Chloride (PVC) is polymer of vinyl chloride. It has resistance characteristics to chemicals including acids and bases. It can be used for blister packs for meat products (Marsh & Bugusu, 2007).

Polyvinylidene Chloride (PVDC) is a homopolymer of vinylidene chloride. Eventhough the structure of this polymer is similar to PVC, there is an additional double chlorine substitution PVDC. It resists chemicals, moreover it shows low water, gas, aroma, and flavor permeability, and high strength (Driscoll & Rahman, 2007) .

Polyamides (PA) are usually produced by hydrolytic or anionic polymerisation of caprolactam. This form of polyamides known as PA6. There are other form of polyamide as PA66, formed by polycondensation of 1,6-diamino hexane and adipic acid. PAs are used as gas barrier in multilayer packaging for meat, fish or cheese. Moreover, it is commonly used as sausage casings and cooking utensils including spoons and spatulas due to its higher melting point (Heimrich, et al., 2012)

Multilayer Formulations

Plastic materials are either made up from one type of polymer called as monolayer plastics or from different layers of plastics held together by adhesives, called as multilayers. Despite of glass and metal materials, plastics do not provide a total barrier to gases, water vapor, and aromas. Therefore plastics are often combined in layers to increase the functionality of the final product (Krochta, 2007). Plastic paper, and/or aluminum can be combined to have efficient functions which is not possible with a single layer structure. Multilayer structures can be produced by polymer coating to a paperboard, lamination two or more plastic with an adhesive and co-extrusion. Layer levels in the multilayer materials were changing between 2 and 10. Examples for multilayer composition can be given as PE/PE/PE, PA/PP, PET/PE/PET, EVOH/PP/PP/PP/PE (Selke, 2005).

Mieth, et al. (2016) lists the most common polymers in multilayer packaging materials, their functions in the packaging and some applications. Polyethylene is used as heat sealable food contact layer moisture barrier which can be combined with gas/aroma barriers such as polyamide. Polypropylene moisture barrier to provide mechanical strength can be a coated with heat seal coatings (PVDC) can be combined with gas/aroma barriers such as PA. Polyethylene terephthalate (PET) can provide gas/aroma and moisture barrier to provide mechanical strength heat resistance. Polystyrene has a gas permeability and printability properties. Therefore it can be outer surface in the structure and can be combined with gas/aroma barriers (coextruded or laminated) (e.g PS/PVDC/PE). Polycarbonate can serve as heat resistant and moisture barrier with its mechanical strength. Ethylene vinyl alcohol (EVOH) is used in modified atmosphere packaging packing of oxygen-sensitive food. It serves as oxygen barrier and can be sandwiched (coextruded) between PE or PP, in some applications also sandwiched between PET, PA or PS (Mieth, et al., 2016).

Legislation

In the European Union (EU), Regulation (EC) No 1935/2004 sets up the criteria which aims to provide safely use of FCMs and articles (EC, 2004). The Regulation states that individual regulations may be required for different groups of materials including plastics, paper and board, metals and alloys, adhesives, printing inks, etc. can be adopted at EU level.

Plastic materials and articles intended to come into contact with food are regulated by Commission Regulation (EU) No 10/2011. Annex I of Commission Regulation (EU) No 10/2011 contains the Union list of authorized monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids (EU, 2011). Annex I of Commission Regulation (EU) No 10/2011 contains the Union list of authorized monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids. It is indicated that potential health risk from the polymers can be ignored, since their molecular weight are above 1000 Da, therefore they are excreted from the body. It can be important only if non- or incompletely reacted monomers, other starting substances, low molecular weight additives migrates from the plastic food contact material into foods. Therefore (EU) No 10/2011 stated that monomers,

other starting substances and additives should be risk assessed and authorized before their use in the manufacture of plastic materials and articles. There are 885 authorized food contact material substances in the list. These listed substances called as "Intentionally Added Substances (IAS)" can be used to manufacture plastic materials, with the restrictions and specifications established in the list (EU, 2011).

The contamination of foods due to the release of chemicals from packaging materials can be originated from the substances used in their formulation (IAS) but also from interactions between different ingredients, degradation products or from the presence of impurities in the raw materials (so called "Non Intentionally Added Substances-NIAS"). Since it is impossible to list and consider all impurities in the authorization, EU 10/2010 gives the responsibility to business operator for risk analysis and taking further risks. Therefore they may be present in the material or article but not included in the Union list (EU, 2011). Substances used in the manufacture of FCMs are regulated with maximum limits that may migrate into foodstuffs without causing any health concerns. There are two migration limit set for plastic based materials and articles: *Specific Migration Limit (SML)* for individual authorized substances fixed on the basis of a toxicological evaluation and *Overall Migration Limit* – 10 mg of substances/dm² of the food contact surface for all substances that can migrate from food contact materials to foods (EU, 2011). Since, overall migration includes all kind of migrated substances, it sets out the general principles of safety and inertness for all Food Contact Materials (FCMs). In Turkey, the Minister of Food, Agriculture, and Animal was released the Turkish Food Codex Communiqué on food contact materials within the frame of compliance with EU (Turk Gıda Kodeksi Tebliği, No:2013/34; No:2012/30, No:2013/35).

Migration Analysis

To determine migrated chemicals into food either for specific or total migration, analysis is performed in food simulants, not actual foodstuffs. Food simulants are used as substitutes for food due to the complexity and variety of foodstuffs, simplification of chemical analysis, and make comparable results between different laboratories.

There are five simulants described in the legislation for plastic (EU 10/2011): 10% ethanol (v/v) in aqueous solution (simulant A), 3% acetic acid (w/v) in aqueous solution (simulant B), 20% ethanol (v/v) in aqueous solution (simulant C), 50% ethanol (v/v) in aqueous solution (simulant D1), vegetable oil (simulant D2), and poly(2,6-diphenyl-p-phenylene oxide, particle size 60-80 mesh, pore size 200 nm, commonly named as Tenax) (simulant E). Food simulants A, B and C are used for hydrophilic foods. Food simulant B is used for acidic foods with pH below 4.5. Food simulant C is used for alcoholic foods with an alcohol content of up to 20 %. Food simulants D1 and D2 are assigned for lipophilic foods. Food simulant D1, D2, and E are used for alcoholic foods with an alcohol content of above 20 %, for fatty foods, and for dry foods, respectively (EU, 2011).

Migration experiments shall be conducted under standardized conditions of contact time and temperature to get the worst scenario for the labelling information on the maximum temperature of use.

Total/Overall Migration Analysis

Overall migration analysis are based on gravimetric methods. For the determination of OM, the corresponding CEN standards are EN 1186 series (EN 1186 1-15). The representative for time and temperature conditions are provided in the Annex III (selection of

proper food simulant) and V (time/temperature conditions) of the Regulation EU 10/2010 (EU, 2011; EN, 1186-1:2002- EN 13130-1:2004).

Specific Migration Analysis

Specific migration analysis are based on chromatographic methods. Therefore, the analytical steps includes extraction and sample clean-up. The general information about specific migration are provided in EN 13130 series Part 1 (Kassouf, et al., 2013). The representative time and temperature conditions are provided in the Annex IV of the Regulation EU 10/2010 (EU, 2011). Simeneau (2008) summarized the official methods used for the specific migration analysis. Gas chromatography equipped with mass spectrophotometry (GC-MS), high pressure liquid chromatography, GC-MS equipped with head space and liquid chromatography-quadruple- time of flight, high pressure liquid chromatography (HPLC) equipped with diode array and fluorescence detector are the common sophisticated equipment used for specific migration. Headspace solid phase micro extraction (HS–SPME) coupled to gas chromatography/mass spectrometry (GC–MS) can be used for volatile substances (Heimrich, et al., 2013). HPLC with chemiluminescent nitrogen detection was used to determine cyclic oligomer of capralactam (Darowska, et al., 2003).

Migration Studies on IAS and NIAS

It has been known that the technology of PET bottle production causes thermal degradation of the polymer and this process can lead to aldehyde formation. Bach et al. (2013) studied the aldehyde contaminations to mineral water stored in PET bottles. It was reported that the concentration of acetaldehyde in water stored in PET bottles changed with the base the concentration of acetaldehyde in PET material.

Carneado et al. (2016) also investigated the migration of aldehydes as well as trace metals and other compounds in to water bottled in PET type plastics and stored at 40, 50, and 60 ^{0}C.

It was observed that, migration of formaldehyde, acetaldehyde and antimony (Sb) were bound on the temperature and the presence of CO_2. Moreover, a degradation compound of phenolic antioxidants as 2,4-di-tert-butylphenol was detected.

Migration of antimony trioxide (Sb), widely used in the polycondensation reaction step of PET bottles as a catalyst in to water stored at 4 and 20 ^{0}C was found as insignificant. However its concentration was increased if the storage temperature increased to 60 ^{0}C (Chapa-Martínez, et al., 2016). Fang et al. (2017) concluded that migration of antimony increased with the storage temperature as well as pH of the water.

The other study reported by Ohno et al. (2001) reveals that extrusion, storage, UV treatments, and sunlight exposure significantly affected concentrations of Irgafos 168 and the degradation products from PP. 2,4-Di-*terts*-butylphenol was the major degradation product produced by UV irradiation, but tris (2,4-di-*tert*-butylphenol) phosphate was the major degradation product produced by extrusion, storage, and sunlight exposure. On the other hand it was concluded that the degradation products showed little health risk.

During the polymerization process of PS, styrene oligomers may occur. On the other hand, Nakaia et al. (2014) concluded that these substances have no estrogenic activity. The findings by Marć & Zabiegała (2017) supported that as well. They stated that the risk of the genotoxicity of styrene oligomers that migrate from polystyrene food packaging into food is very low.

Disposable containers with PS lids are used for hot beverages including coffee and tea. Those types of lids have been reported to release low molecular weight organic compounds

such as styrene and ethylbenzene into the gaseous phase causing direct exposure of consumers to these compounds during drinking (Biedermann-Brem, et al., 2008).

Bisphenol A (BPA) is the manomer of PC. Specific migration limit of BPA was set by The European Commission as 600 μg/kg food (EU, 2011). The studies showed that BPA is an endocrine distributor (Krishnan et al. 1993). There are valuable studies which proved that small amounts of BPA migrates from polycarbonate material into foods (Brede, et al., 2003-Guart, et al., 2014). Based on those type of studies, European Union forbidden the use of PC feeding bottles for infants (EU, 2011).

Fang, et al. (2017) reported that BPA was detected in 77% of samples (40 water sample in PC bottles). After one year storage of the same samples, BPA was detected in % 60 of the fresh water. It was concluded that Bisphenol A may originated from the non-polymerized BPA during PC production and degradation of the PC during the reused of the bottles.

Drowska et al. (2003) highlighted that capralactam, cyclic monomer of PA and its oligomers are the major migrating substances from the materials. Their concentration increased in final product of PA6 after the thermal extrusion of the PA6 film from a granulate. They proved that 95% ethanol and isooctane were not suitable as D2 substitutes for overall migration analysis.

Plasticizers are used in plastic production to increase the functionality of the material by improving plasticity, durability, fluidity, flexibility [54]. There are only five Phthalate plasticizers, namely di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), benzyl-butylphthalate (BBP), –di-isononylphthalate (DINP), di-isododecylphthalate (DIDP), which are allowed to be used in plastics with restrictions (EU, 2011). They are commonly used for PVC. On the other hand, it finds application in PP production as well.

Therefore, Fang et al. (2017) studied to determine the level of phthalate migration from polypropylene food containers into food simulants under different heating time at microwave oven. Their findings indicated that pH as well as heating time affect DEHP and DBP migration. Yang et al. (2016) concluded that the concentration of DEHP and DBP was highest under strong acidity and prolonged heating time. Moreover the highest migration of DBP from the PP food container exceeded specific migration limit.

CONCLUSION

Chemical constituents from all type of food contact material may migrate in to foods in small amounts when they contact with certain type of food. This type of transfer is called as chemical migration. The migrated chemicals from packaging materials can be originated from the substances used in their formulation and also from interactions between different ingredients, degradation products or from the presence of impurities in the raw materials. Substances used in the manufacture of the food contact materials are regulated with maximum limits that may migrate into foodstuffs without causing any health concerns. The recent concern of the scientists are to define and evaluate the toxicities of the specific migrated chemicals including non-intentionally added chemicals (NIAS) rather than evaluation of total migration. However, there are some challenges which is needed to be solved by scientist. Those includes the lack of commercial reference standards and a need for rugged multi residue method. Moreover, more studies are needed to find out the effect of combination of different type of plastics as multilayer formulations as well as different usage conditions.

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Nutrition for Functionality of Poultry Meat and Egg

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Abstract

Functional foods are foods enriched with single ingredients, which influence 1 or more functions of the consumer in a favorable way, exceeding the effects of the normal adequate nutrition. Consumers can expect health from these products. To production of functional poultry meat and egg are attached importance to results related to omega-3 fatty acids, antioxidants, selenium and phenolic compounds' supplementation to poultry diets. It is estimated that their productions will be increased in the future although the food market share of functionel poultry meat and egg is small. In this article, nutrition applications related to functional poultry meat and egg and the physiological, economic and legal aspects of these animal products will be mentioned.

Keywords: Antioxidants, broiler, egg, functionality, laying hens, meat, omega-3

INTRODUCTION

Despite the rapidly increasing world population, chronic diseases such as cardiovascular diseases, cancer, diabetes, obesity and high treatment costs of these diseases, awareness development of the relationship between consumers' health and nutrition, changes in food marketing have led to the emergence of functional foods (Kanberoglu & Meral, 2013). Functional foods described as foods of the today and future are addressed under the healthy living programs and levels of production and consumption is increasing rapidly throughout the world. According to the functional foods commissions' definition of European Union, the functional foods are similar to the traditional foods in point of basic nutritional impact and must be effective in the improvement of human health as both general and physical status and in the preventing of the occurence of diseases (Zduńczyk & Jankowski, 2013). According to 2008 data of functional food market, United States (US) had the largest share while followed by the European Union (EU) countries and Japan. Functional food market in Europea (Germany, France, UK, Netherlands) is being exceeded $ 2 billion and less than 1 % of the total food market. On the other hand, this rate is expected to reach 25 % in the future 10 years. Data for 2015 years showed that the functional food market in Turkey has a volume of 2.2 million dollars, Turkey funtional food market is appeared to be in its infancy according to the functional food market in Japan and EU (Kanberoglu & Meral, 2013).

In recent years, the most commonly studied anmal protein sources on the scope of functional animal food production in Turkey is reported that poultry meat and eggs (Zduńczyk & Jankowski, 2013).

The enrichment of poultry meat and egg by the beneficial substances (omega-3 polyunsaturated fatty acids (PUFAs), conjugated linoleic acid, selenium and antioxidants) for human health leads to an increase at the consumption as the funtional food sources by humans (Grashorn, 2007).

Nutrition Applications for Functionality of Poultry Products

The functionality for poultry products can be gained during the production of raw materials (meat and eggs) and during processing of meat. To design of functional poultry products, functional ingredients such as omega-3 PUFAs, conjugated linoleic acid, vitamin E, selenium, plant extracts and polyphenolic compounds are supplemented to the poultry diets. Direct fortification is obtained by the use of functional feed additives such as plant proteins, whey, fibre, herbs, prebiotics, probiotics, minerals and vitamins during meat processing (Zduńczyk & Jankowski, 2013).

Omega-3 PUFAs

The omega-3 PUFA level and the ration of omega-6 to omega-3 fatty acids in human diets had an important effect on it's metabolism. Daily intake of omega-3 PUFAs is recommended as 450-500 mg for human health (Givens, 2009). It is reported that the high intake of omega-6 PUFAs in relation to omega-3 PUFAs leads pathological changes and several chronic diseases in humans (Grashorn, 2007). The optimal omega-6/omega-3 PUFAs ratio to remove the negative effects of high intake of omega-6 PUFAs on human health should be 1:1 or 2:1 and not exceeded 4:1 (Simopoulos, 2011). To enrich of poultry meat and egg yolks, feedstuffs rich in omega-3 PUFAs such as fish meal, fish oil, flaxseed and flaxseed oil were supplemented to broiler' and laying hens' diets (Zduńczyk & Jankowski, 2013). As a result of this, omega-3 PUFAs are obtained from poultry meat and eggs as animal protein sources. Rahimi et al. (2011) reported that the supplementation of flaxseed and canola seed to broiler diets increased the omega-3 PUFAs levels and decreased the ratio of omega-6 to omega-3 PUFAs of its meat. The feeding the diet containing 3.5 % fish oil for 4 weeks increased four times the omega-3 PUFAs level in egg yolk compared to the control group (Van Elswyk, 1997). At a previous study, the addition of fish oil alone or in combination with flaxseed oil to diets leads to 1.25-1.52 of the ratio of omega-6 to omega-3 in eggs, on the other hand, this ratio is obtained as 25.75 when the diet contained sunflower oil (Farrell, 1998). Especially, the use of fish oil in the poultry diet for enrichment causes off-flavors due to fish-typical substances deposited in broiler meat and laying hens' eggs. The use of flaxseed oil and echium oil in poultry diet is prefered instead of fish oil due to its above mentioned negative effects (Zduńczyk & Jankowski, 2013) because PUFAs found in these oils were effectively transferred from diet into meat and egg (Kitessa & Young, 2009; Sosin-Bzducha & Krawczyk, 2012).

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) and omega-3 PUFAs have similar benefits on the human health although egg yolk and meat of poultry contain small amounts of CLA (0.6- 0.9 mg/g fat) (Zduńczyk & Jankowski, 2013).

A previous study showed that CLA supplementation to broiler diets (2 and 4 %) increased the CLA content of breast and thigh meat by 46 and 38 times compared to the control group, on the other hand, did not affect their fatty acid contents (Sirri et al. 2003). It is reported that breast and thigh meat only meet 3.5 and 9.0 % of human recommended daily intake for CLA in this research, respectively. Enrichment of CLA in tissue is not effective as the enrichment of omega-3 PUFAs as.

Antioxidants

The enrichment of poultry meat or egg with omega-3 PUFAs increased lipid oxidation. The important antioxidants used to prevent lipid oxidation in poultry products are α-tocopherol, selenium, α-lipoic acid and plant extracts etc.

Kim *et al.* (2006) indicated that increasing the level of dietary α-tocopherol (200 or 400 IU) enhanced α-tocopherol concentration and decreased lipid oxidation of of breast and thigh meat of broilers.

Jang *et al.* (2010) found that supplementing poultry diet with quercetin at 200 mg/kg diet substantially decreased TBARS of broiler meat.

Taulescu *et al.* (2011) showed that the addition of selenium (0.3 mg/kg sodium selenite) and vitamin E (200 mg/kg α-tocopherol acetate) to broiler diets enriched with omega-3 PUFAs (15 % flaxseed) significantly decreased the lipid oxidation of meat during storage.

The most effective dose of dietary oregano essential oil supplementation to reduce lipid oxidation was found at 100 mg/kg level in chicken meat (Velasco and Williams, 2011).

Zduńczyk *et al.* (2011) found that the supplementation of the different levels of selenium (0.15 or 50 mg/kg) and vitamin E (40 to 200 mg/kg) increased contents of both anti-oxidants in their breast muscle, i.e. in the case of selenium – from 0.05 mg/kg to 0.08 mg/kg, and in the case of vitamin E – from 3.12 to as much as 14.97 mg/kg and demonstrated to contain a relatively high content of PUFA n-3 (4.84–5.25%).

Sohaib *et al.* (2013) pointed out that dietary supplementation of α-lipoic acid at 150 mg/kg with α-tocopherol acetate at 200 mg/kg increased the antioxidant potential and lipid stability of broiler leg meat and meat products.

Kim *et al.* (2014) also showed that α-tocopherol alone or in combination with red ginseng enhanced the DPPH radical scavenging capacity of broiler meat.

Sohaib *et al.* (2015) reported that supplementation of quercetin with α-tocopherol to broiler diets (especially 300 mg/kg quercetin+300 mg/kg α-tocopherol) improved antioxidant capacity, stability of lipids and fatty acid composition of breast meat of broilers.

Status of Functional Food in Europea and Turkey

The regulations govering the market introduction of foods having nutritional and heath claims are laid down by Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20.12.2006 on nutrition and health claim made on food. The results of research studies have been transferred to practical applications. One of these applications is the production of Columbus® eggs enriched with omega-3 PUFAs and vitamin E that are produced over 50 million per year in Europea (Siro et al. 2010).

It is reported that the main sources of omega-3 PUFAs are raw and processed meat that constitutes 40 % share of poultry meat (Zduńczyk & Jankowski, 2013). According to Givens (2009), the main sources of EPA and DHA are eggs (54.3 mg) and poultry meat (74.8 mg). On the other hand, factors restricting the modification of omega-3 PUFAs content of poultry are deterioration of meat's sensory attributes, higher production costs, the alternative methods of modifying the nutritional value of meat and legal restrictions applicable to the food industry (Zduńczyk & Jankowski, 2013). In line with the provisions of Regulation (EC) No. 1924/2006, for meat to be labelled as a source of omega-3 PUFAs, it has to contain 0.3 g α-linoleic acid/100 g of the product (3 mg/g).

Factors Restricting the Modification of Poultry Meat and Egg and Their Legal Aspects

At the applications for functionality of poultry meat and egg, there are various methods and legal restrictions as well as their high production cost. Being new of functionality concept and the lack of regulation on the issue of functional foods lead to various problems during their market introduction in the world and Turkey despite a rapid increase in the research and investment made on functional foods. Functional foods at different categories and under license are presented to the market and paid attention to have misleading information in labeling due to the absence of regulations for functional food production in the US and EU countries. In Turkey, the market share of functional poultry products (egg rich in DHA, Se or omega-3 PUFAs) is quitely low although there is not it legal size. Functional foods in Turkey are produced within Turkish Food Codex and Food Production with the approval of the Ministry of Food, Agriculture and Animal and the studies are carried out in parallel with the EU harmonization process. In particular, functional poultry products before their introduction to consumption should be determined by clinical tests that contained the protection of functional foods from which diseases and should be written on the product's label.

CONCLUSION

Being conscious for health of consurmers increases day by day the interest for functional poultry meat and egg. Morever, the importance of these animal products is widely understand. The number and variety of these commercial products are increased recently. Poultry meat and egg can effectively be enriched with omega-3 PUFAs, α-tocopherol and Se to meet the requirements for functional foods, whereas enrichment with CLA is less effective. Functional foods will be popular in the future years with numerous practical applications although the share in the functional foods market is low due to their limited studies and high price.

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Starch Based Sugar; Production, Usage and Health Effect

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Abstract

There are two types of sugar production as a sucrose based (sugar beet and/or cane) and a starch based (starch based sugar, SBS). SBS has two fundamental groups, which are glucose syrup and high fructose syrup (HFS). Corn is used as raw material in the production of SBS. Potato, rice and cassava also tried as raw material beside corn. The syrup is obtained from the hydrolysis of starch to glucose, maltose, maltotriose and dextrins. Enzymatic and acid hydrolyses have been used traditionally to modify native starches and to create glucose syrup. HFS is produced by the hydrolysis of starch into glucose followed by isomerization into fructose. Therefore, glucose syrup is an intermediate in HFS production. Three forms of HFS are commercially available: HFS-42, HFS-55 and HFS-90. Syrup is used in foods to soften texture, add volume, prevent crystallization and to enhance flavor. SBS is also used with sucrose based sugar in food products to enhance their own effect. There is a lot of argument on SBS negative health effect but there is no proved scientific study. In biochemistry view there is no difference between sugar content, calorie value of glucose syrup and fructose syrup. Metabolism of glucose and glucose syrup are same but for HFS there is no regulation by insulin so fructose metabolism rapid and uncontrollable in the body. SBS is one of the important food intermediate product and can be used to enhance desirable properties of food. Safe and quality syrups can be obtained by food industry but there still needs more study for effects of SBS on human body.

Keywords: Starch based sugar, high fructose syrup, glucose syrup, sucrose

INTRODUCTION

Starch based sugars (SBS) obtained from starch containing plants are the second largest important sweetener in the world. Starch based sugars are viscous liquids consisting primarily of a solution of sugar in water containing large amount of dissolved sugars but showing little tendency to deposit crystals (Sugar Institution, 2017). The viscosity arises from multiple hydrogen bonds between the dissolved sugar, which has many hydroxyl (OH) groups, and the syrups can be either medicinal or nonmedicinal. Glucose syrup is food syrup made from the hydrolysis of starch. It can also be described as aqueous solution of glucose, maltose and other nutritive saccharides from edible starch. Glucose syrup is produced from corn also made from other starch crops including potato, wheat, barley, rice and cassava (Hull, 2010).

Starch is a natural, renewable and biodegradable biopolymer produced by many plants as a source of stored energy. Starch is found in plant roots, stalks, crop seeds and staple crops such as rice, corn, wheat, tapioca, and potato.

In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. Enzymatic and acid hydrolysis have been used traditionally to modify native starches and to create starch based sugars contribute the

functionality of foods with their properties such as; viscosity, desirable sweetness, good thermal stability, avoidance of ice crystals, decrease the freezing point, emulsion stability, fermentable, gel formation, altered boiling point, increase foam stability, set off the taste, enrichment of the taste, microbial stability, water absorption, osmotic pressure, enhance color, inhibit the crystallization of sugar and nutritious (Foresti et al., 2014).

The transformation of starch into sugar is an important branch of the starch industry and is one of the most important applications of biotechnology. Countless foods contain ingredients produced by the breakdown of starch. Under the right conditions, starch molecules can be broken down into sugar. This process makes it possible to obtain sugar from the starch of many different plants, rather than just sugar beets or sugar cane. This is now being done by industrial-scale starch saccharification. The most important sources of starch are maize, potatoes, and wheat (Chandrasekaran M., 2015).

Syrup is used in foods to soften texture, add volume, prevent crystallization of sugar and enhance flavor. They are also used in large quantities in fruits, liquors, crystallized fruits, bakery products, pharmaceuticals and brewery products. Depending on the method used to hydrolyze starch and on the extent to which the hydrolysis reaction has been allowed to proceed, different grades of glucose syrup are produced, which have different characteristics and uses. Due to their high osmotic pressure, low water activity and high temperature processing, they are usually resistant to bacterial spoilage. However some spoilage microorganisms, such as yeast and mold spores, can survive in the syrup and still grow under storage conditions (Eke-Ejiofor, 2015).

Fructose-based syrups are attractive industrial sweeteners due to the fact that they are sweeter than sucrose and cost less to produce. High Fructose Syrup (HFS) is produced by enzymatic isomerisation from glucose syrup.

Production of Glucose and High Fructose Corn Syrup

There are five starch based sugar (SBS) producer company in Turkey, all of which are privately owned and have a total processing capacity of 1 MMT of corn. Only domestically grown corn starch is used for obtaining SBS in Turkey (Sugar Institution, 2017).

Starch is the major raw material for the production of glucose syrup and it is widely used in the food and pharmaceutical industries. Enzymatic and acid hydrolyses have been used traditionally to modify native starches and to create products with altered solubility, viscosity, and/or gelation properties that find broad applications in food, paper, textile, and other industries (Eke-Ejiofor, 2015).

Syrup is obtained from the hydrolysis of starch to glucose, maltose, maltotriose and dextrins using chemicals (caustic soda, hydrochloric acid) and enzymes (α-amylase and glucoamylase) to hydrolyze starch to syrup containing mostly glucose. α-amylase is an endo-specific enzyme that randomly catalyzes the hydrolysis of α -(1→4) glycosidic linkages in amylose and amylopectin chains.

In the last years, α -amylase-catalyzed hydrolysis of starch has received much attention due to its industrial value for the production of glucose and fructose syrups and other starch hydrolysates (Foresti et al., 2014).

There are three stages in the conversion of starch: gelatinisation, involving the dissolution of the nanogram-sized starch granules to form a viscous suspension; liquefaction, involving the partial hydrolysis of the starch, with concomitant loss in viscosity; and saccharification, involving the production of glucose and maltose by further hydrolysis (Chaplin and Bucke, 1990).

Depending on the method used to hydrolyze starch and on the extent to which the hydrolysis reaction has been allowed to proceed, different grades of glucose syrups are

produced, which have different characteristics and uses. The syrups are broadly categorized according to their dextrose equivalent (DE). The term dextrose equivalent (DE) can be regarded as an indication of how far the conversion process from starch to dextrose has gone. Starch has a DE value of zero, whilst dextrose, the final end product of starch hydrolysis, has a DE value of 100. DE is a measure of the total reducing sugar present in syrup. The syrups are broadly categorized according to their DE. In the food industry syrups are generally known as glucose 60DE and glucose 38, 40, 42 DE (Hull, 2010).

HFS is produced by the hydrolysis of starch into glucose followed by isomerization into fructose. Another enzyme (i.e. glucose isomerase) is used to isomerize glucose in corn syrup to fructose to yield HFS. Therefore, glucose syrup which is also referred to as dextrose syrup, is an intermediate in HFS production. HFS products classified according to their fructose content: HFCS-90 (90% fructose and 10% glucose), HFCS-42 (42% fructose and 58% glucose), and HFCS-55 (55% fructose and 45% glucose). HFCS-90 is the major product of these chemical reactions and is blended with glucose syrup to obtain HFCS-42 and HFCS-55.

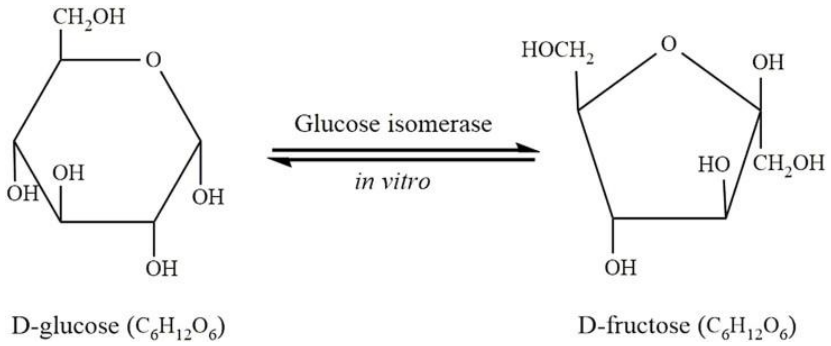

Figure. 1. Isomerization of D-glucose to D-fructose using glucose isomerase (Jia et al., 2017)

Physicochemical properties of SBS and effect on the product quality

Physico-chemical properties such as moisture content, mineral ash, total available carbohydrate (TAC), total titratable acidity (TTA), pH and dextrose equivalent (DE) are important parameters for SBS. State behavior such as crystallization and glass transition are important properties of SBS. The glass transition temperature (Tg) plays a critical role in many food product's quality and storage stability.

The understanding of glass transitions of food systems has allowed food material characterization and prediction of their behavior at high solids contents and in the frozen state at varying temperatures and water contents. State diagrams, or supplemented phase diagrams, provide useful maps for the observation of changes in glass transition as a function of water content or varying levels of freeze-concentration. (Chen et.al., 2015).

Sucrose crystallization is encountered in many food and pharmaceutical applications. Food products where sucrose crystallization is important include refined sugar, confections, ready to eat cereals, and some snack foods. During processing, nuclei are either formed in situ or added as seeds. Once formed or added, crystal grow at a rate dependent on conditions in their surrounding environment in a series of steps.

Health concern of SBS

Many foods undergo thermal processing during its industrial production in order to improve the quality and stability. However, thermal processing of foods with high sugar

contents can promote the formation of undesirable compounds such as 5-hydroxymethyl-2-furfural (HMF). This contaminant is practically absent in fresh foods, but its content rises naturally during long periods of storage or after severe heat treatment. Several studies report that high concentrations of this contaminant may have cytotoxic effects in the respiratory tract and may cause irritation of the eyes, skin and mucous membranes.

Over the past four decades, the prevalence of health disorders, including hypertension, obesity, metabolic syndrome, diabetes, and kidney disease, has drastically increased. In parallel to the dramatic rise in the prevalence of these cardio renal diseases, a similar increase in the consumption of fructose has occurred. Recent studies have implicated excessive fructose intake as one of the factors driving the increases in these health disorders (Le et al., 2012). The high consumption of foods added with corn syrups has been associated with several metabolic disorders such as diabetes, obesity and heart problems, showing the concern by different researchers. Recently a growing search has been observed for new natural sweeteners that can offer benefits to health due to the presence of bioactive functional molecules (minerals, vitamins and polyphenols) (Andrade et.al., 2016).

The rise in obesity that has occurred since the introduction of HFCS into the diet suggested a link between the two. Fructose, unlike glucose, does not stimulate insulin secretion from the pancreatic B cells (Melanson et al., 2007). There is no regulation for the fructose by insulin so fructose metabolism rapid and uncontrollable in the body. And also fructose goes right to the liver and it can be triggers lipogenesis (the production of fats like triglycerides and cholesterol) (Rippe and Angelopoulos, 2013). However, many have refuted the conjecture that HFCS alone is at fault, suggesting that sugars in general are the problem. Some studies indicate that HFCS and sucrose elicit similar post-metabolic profiles (Melanson et al., 2008), but there are differences in how these sugars are metabolized and utilized in the body (Bocasly et al., 2010).

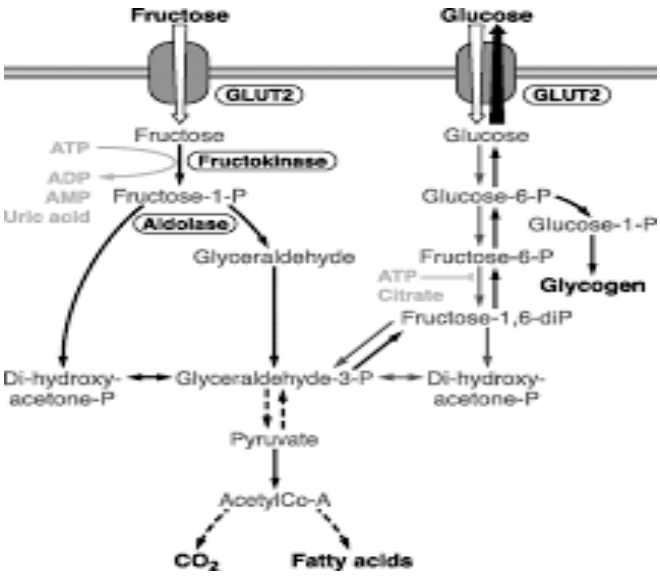

Figure 2. Metabolism of fructose and glucose in the liver (Rippe and Angelopoulos, 2013).

CONCLUSION

As a result, the SBS is one of the important food intermediate product and used to enhance desirable properties of food. There is a lot of argument on SBS negative health effect such as diabetes, obesity and heart problems, but there is no proved scientific study. Safe and

quality syrups can be obtained by food industry but there still needs more study for effects of SBS on human body.

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Optimization of manufacturing parameters used safflower oil obtained by solvent extraction method for determination of oil properties

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Abstract

In this study crude oil from Remzibey-05 safflower seeds has been breeding in Turkey, was obtained by solvent extraction method. The maximum oil extraction rate of 30% was obtained at the end. Safflower oil has high unsaturated fatty acid content (91.17%) and highest fatty acids found was linoleic (C18:2) 56.82% and oleic (C18:1) acids 33.98%. Physicochemical properties of safflower seed oil were determined with the analysis and measurements. In addition, solvent-type (n-hexane, and dichloromethane diethyl ether), seed, seed-to-solvent ratio: ratio of solvent mixture (1:1, 1:2 and 1:3 (w/w)), stirring rate (200,400 and 600 rpm) and extraction time (1, 2 and 3 hour), the parameters of fat synthesis from solvent extraction method, which determined the effect of experimental design. According to the results of the ANOVA analysis of variance, the parameter that the highest degree of influence the production of safflower oil seed-to-solvent ratio, while the lowest effective parameter was extraction time.

Keywords: Fatty acids, Oil characteristics, Safflower seed, Solvent extraction, Taguchi method

INTRODUCTION

As a member of the family *Compositeae* safflower (*Carthamus tinctorius* L.) is a one year plant. Today, safflower is primarily cultivated for its oil, which is used for food and industrial purposes. As well as using in oil, margarine, mayonnaise and salad oil production, because of its high proportion of linoleic acid is also used in paint and varnish industry. Safflower oil is tocopherol (vitamin E) rich. The amount of the total tocopherol in seed can be up to 400 mg kg^{-1} (Weiss, 2000). After obtaining the remaining portion of the oil, cake contains between 22-24% protein and that makes it a good animal fodder.

Safflower is a minor crop with a world production of about 650,000 tons in 2009 (Weiss, 2000). The world's total plantation area of safflower is 1.121212 ha. India is in the first ranks with area of 700,000 ha and 400,000 tons of production and meets about 70% of world production of safflower. The United States, Mexico, Argentina, Kazakhstan and China were the followers. Approximately 95% of world production of safflower, carried out in these countries. In Turkey, only 165 hectares to 150 tons of seed are produced (Weiss, 2000).

Seed production of safflower is much less in Turkey, so it cannot be processed as a vegetable oil. Safflower can be evaluated as one of the alternative products especially in relatively dry agricultural areas, because of a high tolerance to drought and salinity. Thanks to the drought-resistance the importance of safflower seed is expected to increase due to

breeding problems caused by global warming nowadays. The safflower seed oil content is increased thanks to breeding programs. After turning off edible oil deficit in countries, oil crops which has to be evaluated renewable energy sources and raw materials for the production of oil for biodiesel as the environmentally friendly fuel. Each country should produce oil-seed plants high in fat to ensure continuous production of biodiesel and in accordance with their own local conditions. This is why alternative oil plants as well as the rehabilitation work of existing oil crops are needed to increase oil rates.

In our country, three kinds of safflower seed including Remzibey-05, Yenice and Dinçer are planted. The purpose of this study optimizing the parameters used in solvent extraction of Remzibey-05 such as; the type of solvent (n-hexane, and dichloromethane diethyl ether), seed: ratio of solvent mixture (1:1, 1:2 and 1:3 (w/w)), stirring rate (200, 400 and 600 rpm) and extraction time, and determining the physicochemical properties and fatty acid composition are aimed.

MATERIAL and METHODS

Materials

Safflower seeds (SS) of Remzibey-05 cultivars (*Carthamus tinctorius L.*), were obtained from Agricultural Research Institute in Eskişehir. While determining the physicochemical properties and analysis, as a solvent; n-hexane (Labkim, analytical grade 96%), diethyl ether (purex analytical grade 99.7%) and dichloromethane (HPLC grade 99.5%) was used. Electric mill is used for grinding the seeds, BUCHI rotary vacuum evaporator (Rotavapor R-210), vacuum pump (V-700), HEIDOLPH RZR 2021 mechanical Agitator and NUVE mark NF400 type centrifugation was used in extraction process.

Methods

Remzibey-05 type safflower seeds used in the experiments are dried at 120 ^{o}C for 1 hour. Parametric studies of 100 g of ground safflower seed, extraction with n-hexane, diethyl ether and dichloromethane 1:1, 1:2 and 1:3 (w/w), seed: solvent mixing ratio, 200, 400 and 600 rpm stirring rate 1, 2 and 3 hour oil extraction times were obtained. Milled seeds are taken to rotary evaporator glass flask and mixed with the rates defined for different types of solvent.

At the end of extraction, the mixture filtered after being separated and the remaining portion of the seed-cake mixed with solvent and oil from the evaporator again under vacuum. Then, core centrifuged at centrifugal separator 4200 rpm and 3600 RFC for 45 minute. Particles remaining in suspension in oil are precipitated.

Fatty acid composition and Physicochemical Properties

The density was measured with a densimeter. The color was determined by Lovibond method of AOCS, Cc 13e-92 (AOCS, 1994) using a glass cell with an optical path length of 153 mm with PFX 880 Tintometer.

The pH value was measured with a digital pH meter (Hanna pH211). Dry matter was determined by oven drying at 105 ^{o}C to the constant weight (AOAC, 1990). FFA content, peroxide value (POV) and iodine value (IV) were determined using AOCS methods, Ca 5a-40 (AOCS, 1989), Cd 8-53 (AOCS, 1997) and Cd 1-25 (AOCS, 1993), respectively. The degree of unsaturation (DU) was determined as described by Porzucek and Raznikiewicz (1990)

using the following equation with computed values obtained from GLC after comparison with reference standards:

$$DU = \frac{1\times(\%wt\ MUFA)+2\times(\%wt\ DUFA)+3\times(\%wt\ PUFA)}{100}$$

where: MUFA – represents monounsaturated fatty acid; DUFA – diunsaturated fatty acid and PUFA – polyunsaturated fatty acid.

The refractive index was determined using an Abbe refractometer (WYA Abbe refractometer, Ningbo Yuda Import & Export Co. Ltd, China) at 20 ^{o}C(AOAC, 1990). Fatty acid methyl esters (FAMEs) were prepared from the oil samples according to a laboratory protocol described previously (Yu et al., 2002; Lutterodt et al., 2011). Individual fatty acid methyl esters were identified by comparing their retention times with those of FAME standards. Area under each fatty acid peak relative to the total area of all fatty acid peaks was used to quantify the fatty acids identified. Results are reported as g fatty acid 100 g^{-1} total fatty acids (Lutterodt et al., 2011). All samples were analyzed in duplicate.

Taguchi Design

The Taguchi optimization method was used for the process optimization of solvent extraction of safflower seeds. Taguchi method was employed to reduce the number of experiments and improve the performance characteristics. This method uses special orthogonal arrays to study all the design parameters using a minimum number of experiments. Orthogonal array means that parameters can be evaluated independently of one another; the effect of one parameter does not interfere with the estimation of the influence of another parameter (Ross, 1989). In Taguchi method, the signal-to-noise (S/N) ratio is used to measure the quality characteristics deviating from the desired value. In the present study, three levels are defined for each of the parameters and a L9 orthogonal array scheme was adapted, which required nine experiments instead of 27 individual experiments to complete the process. After that the results were converted into S/N ratio data. In order to more systematically perform an analysis of the relative importance of each parameter, an analysis of variance (ANOVA) was used to optimize the results obtained using Taguchi method.The four selected parameters, at three levels L9 experimentally studied are shown in Table 1.

Table 1. Design experiments for the production of safflower seed oil

Parameters	**Levels**		
	1	**2**	**3**
Seed-to-solvent ratio (A)	1:1	1:2	1:3
Solvent type (B)	n-hexane	Diethyl ether	Dichloromethane
Stirring rate (rpm) (C)	200	400	600
Extraction time (h) (D)	1	2	3

Table 2 is indicate the levels of the parameters. Values represent the average of three analyses.

Table 2. The result of orthogonal test L9

Experiment no	Solvent type	Seeds-to-solvent ratio	Extraction time (h)	Stirring rate	Oil extraction w/w
1	1	1	1	1	18
2	1	2	2	2	24
3	1	3	3	3	30
4	2	1	2	3	27
5	2	2	3	1	21
6	2	3	1	2	28
7	3	1	3	2	14
8	3	2	1	3	16
9	3	3	2	1	25

RESULTS and DISCUSSION

Fatty Acid Composition

Fatty acid composition of safflower seed oil Remzibey-05 is given in Table 3. Content of fatty acids is composed mainly linoleic acid of 56.82%, 33.98% oleic and 5.84% and palmitic acid.

Total saturated fatty acid content was 8.83% and total unsaturated fatty acid content was 91.17%. Mono unsaturated fatty acids were 34.29% and poly unsaturated fatty acids were 56.88%.

Table 3. Fatty acid compositions of safflower seed oil

Fatty acids (%)		Safflower seed oil
Palmitic	(C16:0)	5.84
Margaric	(C17:0)	0.03
Stearic	(C18:0)	2.22
Arachidic	(C20:0)	0.36
Behenic	(C22:0)	0.28
Lignoceric	(C24:0)	0.01
Palmitoleic	(C16:1)	0.09
Oleic	(C18:1)	33.98
Linoleic	(C18:2)	56.82
Linolenic	(C18:3)	0.06
Gadoleic	(C20:1)	0.19
Erucic	(C22:1)	0.01

Physicochemical Properties

Density of safflower oil was 0.819 g mL^{-1}. Free fatty acid content was about 0.28 g $100mL^{-1}$ in terms of oleic acid. Refractive index value of oil was 1.468. Iodine value of safflower oil was 117.87 and higher values are indicators of higher unsaturated fatty acid amounts and obtained data are confirmed with fatty acid compositions further.

Peroxide value of safflower oil was 2.45 meg kg^{-1}, saponification value of samples were 178.33 mgKOH g^{-1}. unsaponifiable matter content was about 1.24 on the other hand pH value of samples were 5.18 (Table 4).

Table 4. Physicochemical properties of safflower seed oil

Parameters	Safflower seed oil
Density at 15 ^{0}C (kg m^{-3})	819
Kinematic viscosity at 40 ^{0}C (mm^2 s^{-1})	16.5
Free fatty acid (% FFA as oleic acid)	0.28
Acid value (mgKOH g^{-1})	0.56
Iodine value	117.87
Peroxide value (meg kg^{-1})	2.45
Refractive index (25 ^{0}C)	1.468
Saponification value (mg KOH g^{-1} oil)	178.33
Unsaponifiable matter (% g g^{-1} oil)	1.24
Colour (5.25")	4R-20Y
Molecular weight (g mol^{-1})	876.30
Percentage oil content in seed (%)	30±0.4
Physical state at room temperature	Liquid
Iron (mg kg^{-1})	1.49
pH	5.18

Optimization of Parameters

The main principle of Taguchi method of experiment, the minimum number of individual and mutual effects of the factors obtained from the research community. Taguchi method is used to test the effectiveness of changes in the S/N ratio was determined by measuring the state of the factors, and safflower oil, is aimed to obtain the maximum amount of oil is better than the largest calculations were performed according to the criteria (Mandal et al., 2008).

Four variables (solvent type, seed-to-solvent ratio, stirring rate and extraction time) at three levels were used to design the experiment in MINITAB 14

Safflower oil solvent extraction method is used to obtain the highest degree of importance to the control parameters, respectively, were as follows: A (seed-to-solvent ratio)> B (solvent type)> C (stirring rate)> D (extraction time). Safflower oil production from the control parameters and their impact on the different levels of the S/N ratio, shown in Figure 1.

Who obtained the maximum amount of safflower oil is the best set of experimental conditions, takes place 90% confidence interval $A_3B_2C_2D_3$. In other words, seed-to-solvent ratio of parameter 3 level (1:3), solvent-type parameter 2 level (diethyl ether), stirring rate, the parameter 2 level (400 rpm) and extraction time of parameter 3 level (3 h) experiments performed and resulted the maximum oil extraction. In these circumstances, at the confirmation test (confirmation experiment) 30 g of safflower seed oil was obtained 100 g seed.

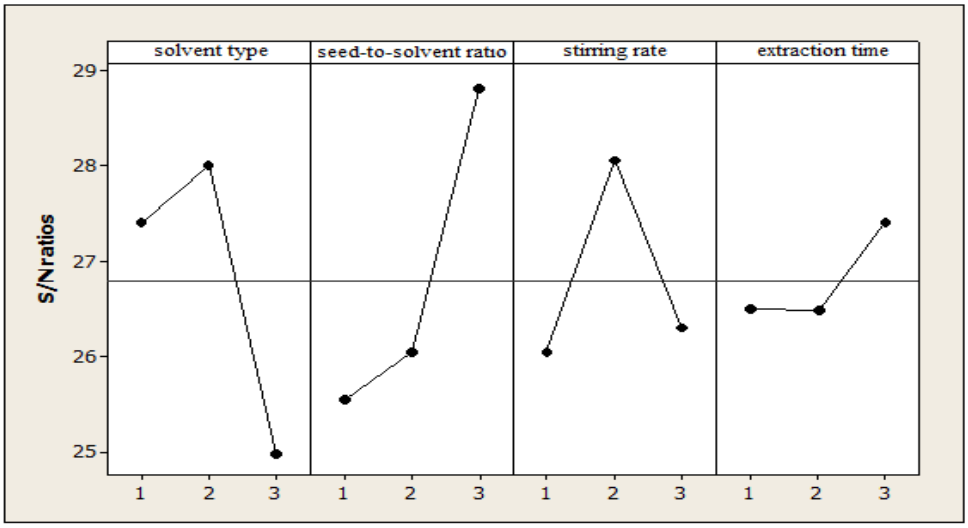

Figure 1. The effects of each parameter at different levels on the yield of safflower oil.

According to the results of the ANOVA analysis of variance, seed-to-solvent ratio of 90% confidence level (confidence interval) is effective. The P value was found to be 0.084. Safflower seed oil production, the percentage of impact of seed-to-solvent ratio, parameter was 35.32%, the percentage of solvent-type parameter effect was 32.72%, stirring rate 21.88% and the percentage of impact parameter, extraction time parameter influence was found to be 10.07%.

In this study, the most influential factors were seed-to-solvent ratio and solvent-type and degrees of impact parameters close to each other. As seen in Figure 1 solvent-type parameter is very little difference between levels 1 and 2, instead of diethyl ether using n-hexane solvent does not alter the result.

CONCLUSION

Safflower oil, edible oil from an examination of its physical and chemical properties due to the human diet, after the oil portion taken off, the remaining pulp was used in animal nutrition. Taguchi experimental design, the minimum number of experiments done by applying the solvent extraction method and the ANOVA analysis of variance with the least amount of safflower oil production will be provided more effective control parameters and the degree of importance of each parameter were determined. Specified conditions to be achieved for production to maximize the amount of fat while the lowest degree of impact parameter is defined as a shortened extraction time will be possible to save time.

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EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Optimization Using Decision Trees Method in Multivariable Food Engineering Experiments and Its Sample of Applicability on Experiment Related with the Nisin Production of *Lactococcus lactis* N8

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Abstract

In this study, the ranges of independent variables resulting the optimum result of experiment should be selected was determined using decision trees method. Thus, the applicability of decision trees method has been proposed to food engineering experiments aiming the optimization. The sample application of the decision tree method proposed in the study was performed in the experiment aiming optimum nisin production of *Lactococcus lactis* N8. According to the findings obtained from the sample application it was observed that the decision trees method determines both optimum variable values and their tolerance ranges. Furthermore, the method proposed was not only determined the optimal ranges of variable values also it was determined the variable ranges for all possible experimental results. Accordingly, at the end of the study, advantages of the proposed method were explained by comparing with similar methods and how the experimental design should be to make the method more effective was proposed.

Keywords: Decision Trees, Modelling, Nisin Production, Optimization, Prediction

INTRODUCTION

In the field of food engineering, experiments are carried out for optimization especially in microbiology and biotechnology experiments (Mandenius et al., 2008; Kalkan et al., 2014). In these optimization experiments, experimental results are investigated depend on the interaction of independent variables and their variation of the variable values. Thus, it is determined what the variable values that maximize or minimize the experiment result should be (Banga, et al., 2003). Many methods especially dependent on curve fitting are used as optimization methods (Kalkan et al., 2016; Saguy et al., 1984). The well-known and most used of these methods is the response surface methodology that is depending on second-order curve fitting. However, even in this method, only the coefficients that produce the optimum result can be determined. Namely the optimal ranges of independent variables that produce the requested result are not provided precisely in the response surface methodology. For this reason, it is not much sensitive to the external factors can change the experimental results (Myres et al, 1995; Baş et al, 2007).

Moreover, if the optimum experimental results are more than one, the current methods can determine only one of them. Therefore, it cannot determine also the variable values that will create the second optimal experimental result. Apart from all these, the number of optimization methods used in the field of food engineering is not much more (Koç et al., 2010).

For this reason, a method that will provide the optimum ranges of independent variable values to produce requested experimental results has been proposed as alternative optimization method in this study. This method proposed was applied to the sample food engineering experiment and tested. According to the findings obtained, advantages were also determined.

MATERIAL and METHOD

Material

In this study, the sample experiment is the nisin production of Lactococcus lactis N8 with hemin-stimulated cell respiration in the fed-batch fermentation system. This experiment was previously performed using response surface methodology and is in the literature (Kördikanlıoğlu, 2014; Kördikanlıoğlu et al, 2015). Thus, the comparison of the proposed method with the surface response methodology can be done by using the data of this experiment. Also, since the same experiment is in the literature, details of the experiment are not given in this study. Variables are glucose, hemin and oxygen concentrations used in fed-batch fermentation. Tried variable values and the results obtained are also shown in the Table 1.

Table 1. Variable value variations used in the sample experiment and the results obtained (Experimental Design used for Optimization of Hemin, Glucose and Dissolved Oxygen Concentration in the Fed-batch Fermentation System)

Experiment No	Glucose (g L^{-1} h^{-1})	Hemin (µg mL^{-1})	Dissolved Oxygen (%)	Nisin (IU mg^{-1})
1	1	1,5	50	1225,33
2	5,5	1,5	50	1153,64
3	1	2,5	20	1138,3
4	1	2,5	80	762,48
5	5,5	2,5	50	1662,53
6	5,5	1,5	50	1101,16
7	10	0,5	20	464,56
8	1	1,5	50	1268,76
9	5,5	1,5	80	314,63
10	10	2,5	80	1271,82
11	10	0,5	80	1346,87
12	1	0,5	20	1212,78
13	5,5	0,5	50	1231,74
14	5,5	1,5	50	1095,84
15	5,5	1,5	50	1073,39
16	5,5	1,5	50	1077,05
17	1	0,5	80	733,13
18	10	2,5	20	1670,88
19	5,5	1,5	20	1191,11
20	5,5	1,5	50	1118,07
21	15	4,5	20	384,4
22	15	4,5	80	491,87
23	5,5	4,5	20	910,42
24	5,5	4,5	80	428,43
25	10	4,5	20	1168,44
26	10	4,5	80	680

Method

Decision Trees Method

Decision trees are a decision support system that is used to determine which variable value variations produce which result values. Decision trees achieve this goal using different mathematical algorithms. In this study, ID3 algorithm based on entropy and information gain is used. This algorithm primarily measures the information gain between the variables and experimental result. Then the variable with highest information gain is divided into clusters containing the same values. Then the same procedure is applied to the remaining variables and subsets are generated. Also determines the variable limits that must be selected to create each of subsets during clustering (Rokach et al., 2014; Ville., 2006; Akben et al., 2016) The information gain between any variable and corresponding result is as in Equation 1. In Equation 1, x is a variable, x_i is the i^{th} value of variable x then z is the experimental result.

$$I(x) = E_z - \sum_{i=1}^{i=n} P(x_i, x) E_{x_i} \quad (1)$$

In the Equation 1, E is the entropy value as in Equation 2 and P is the probability function as in Equation 3.

$$E_{x_i} = -\sum_{c=1}^{c=m} P\left(z_c, \sum z_c\right) \log_2^{P(z_c, \sum z_c)} \quad (2)$$

In the Equation 2, m is the number of result values corresponding to the variable x_i while result values corresponding to the variable x_i are z_c.

$$P(x_i, x) = \frac{\sum x_i}{\sum x} \quad (3)$$

In Equation 3, the sum of each x_i value is divided by the sum of all x values. The calculation of the entropy value for a variable value on the sample experimental design is as in Fig.

$$E_{x_1} = -1/3 \log_2{}^{1/3} - 2/3 \log_2{}^{2/3}$$

$$E_Z = -1/5 \log_2{}^{1/5} - 2/5 \log_2{}^{2/5} - 2/5 \log_2{}^{2/5}$$

$$I(x) = E_Z - 3/5 E_{x_1} - 2/5 E_{x_2}$$

$$\begin{bmatrix} x_1 & y_1 \\ x_1 & y_2 \\ x_1 & y_2 \\ x_2 & y_2 \\ x_2 & y_2 \end{bmatrix} = \begin{bmatrix} z_1 \\ z_2 \\ z_2 \\ z_3 \\ z_3 \end{bmatrix}$$

x_1 is the first value of variable x
x_2 is the second value of variable x

y_1 is the first value of variable y
y_2 is the second values of variable y

z_1 is the first value of experimental result
z_2 is the second value of experimental result
z_3 is the third value of experimental result

Figure 1. Calculation of the entropy value for a variable value on the sample experimental design

In Figure 1, the decision tree result generated in single process for two variables. However, in this study the decision tree was applied separately for each variable since the experimental model results corresponding to the variables were not equal. Thus, the optimal limits for each variable were determined separately.

Curve Fitting Method based on Vandermonde matrix

The polynomial model obtained by curve fitting was used to evaluate the effect of modeling on decision tree use in this study. The polynomial model is based on the Vandermonde matrix. Thus, the polynomial model used can also be called the Vandermonde polynomial (Cazals et al., 2005). The method of creating Vandermonde polynomials is as follows.

Assume that $V_{n,m}$ is the n^{th}value of m^{th}variable and $R_{n,m}$ is the experimental result values corresponding to this variable. In this case, the relation between the $V_{n,m}$ and $R_{n,m}$ is as in Equation 1. Thus, the polynomial model can be represented as is in Equation 2.

$$V_{n,m}{}^{n} + \cdots + c_2 V_{n,m}{}^{2} + c_1 V_{n,m}{}^{1} + c_0 V_{n,m}{}^{0} = R_{n,m} \quad (1)$$

The c_n values in Equation 1 are polynomial coefficients can be calculated as in Equation 2.

$$\begin{bmatrix} V_{0,m}{}^{0} & V_{0,m}{}^{1} & V_{0,m}{}^{2} & \cdots & V_{0,m}{}^{n} \\ V_{1,m}{}^{0} & V_{1,m}{}^{1} & V_{1,m}{}^{2} & \cdots & V_{1,m}{}^{n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ V_{n,m}{}^{0} & V_{n,m}{}^{1} & V_{n,m}{}^{2} & \cdots & V_{n,m}{}^{n} \end{bmatrix} \begin{bmatrix} c_{0,m} \\ c_{1,m} \\ \vdots \\ c_{n,m} \end{bmatrix} = \begin{bmatrix} R_{0,m} \\ R_{1,m} \\ \vdots \\ R_{n,m} \end{bmatrix} \quad (2)$$

In this study, each polynomial equation represents relation between the experimental result and one of variables. For this reason, a different polynomial equation was created for each variable in the study.

RESULTS and DISCUSSION

Decision trees method was tested for both raw variable values and polynomial model in this study. Thus, it is also determined whether modeling is necessary if decision trees method will use. Experiment result values for the test of proposed method were divided into 4 classes for each 500 IU mg-1 interval. That is, each of result values in the range of 0-500, 500-1000-1500, 1500 < IU mg-1 was considered as a single value and experimental result values were assigned as 4 class. This process allows the creation of limit values for each interval. The optimum limits determined by decision trees for both raw and modeled variable values are the as in the Table 2.

Table 2. Optimum limits for both raw and modelled variable values

For Nisin > 1500 IU mg^{-1}	Glucose (g L^{-1} h^{-1})	Hemin (µg mL^{-1})	Dissolved Oxygen (%)
Limits for raw data	">3.25" and " < 15"	" > 2" and " < 3.5"	" > 20" and " < 65"
Optimal values of raw data (Mean of Limits)	9.125	2.75	42.5
Limits for modelled data	">5.83" and " < 11.57"	" > 2.36" and " < 3.64"	" > 31.1" and " < 56.3"
Optimal values of modelled data (Mean of Limits)	8.7	3	43.7

As it is seen in the table, applying the decision trees to experimental data obtained by modeling was produced more applicable (narrower) limit ranges as compared to raw experimental data. If so, it would be more appropriate to apply the decision trees to the experimental results obtained by modeling. The modeling applied in the study is to create the Vandermonde polynomials. Number of the variables values tried in the study was 4 for hemin and glucose and 3 for dissolved oxygen rate. Therefore, the polynomial degree for the hemin and glucose was selected as 3 and for the dissolved oxygen rate was selected as 2. The experimental values produced by the model were normalized to reduce the error rate. That is, in accordance with the actual distribution ratio the calculated result values were placed (stretched) between the largest and smallest experimental result of the raw data. The normalized graphs obtained by polynomial models can be seen from Figure 2.

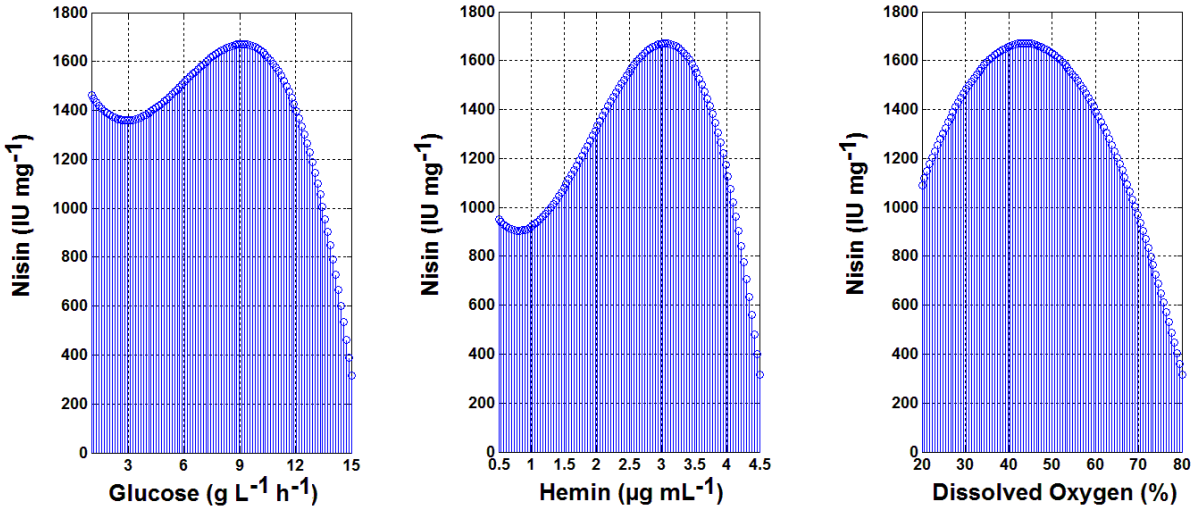

Figure 2. Graphs that obtained by normalized polynomial models.

The limits of optimal variable values calculated by decision trees can be seen in Figure 3. The cyan color cube in Figure 3 is the space that results of the experiment will be nearly optimized while the red dot is precise coordinate of optimal experimental result. Also, the red dot is the center of the cyan color cube.

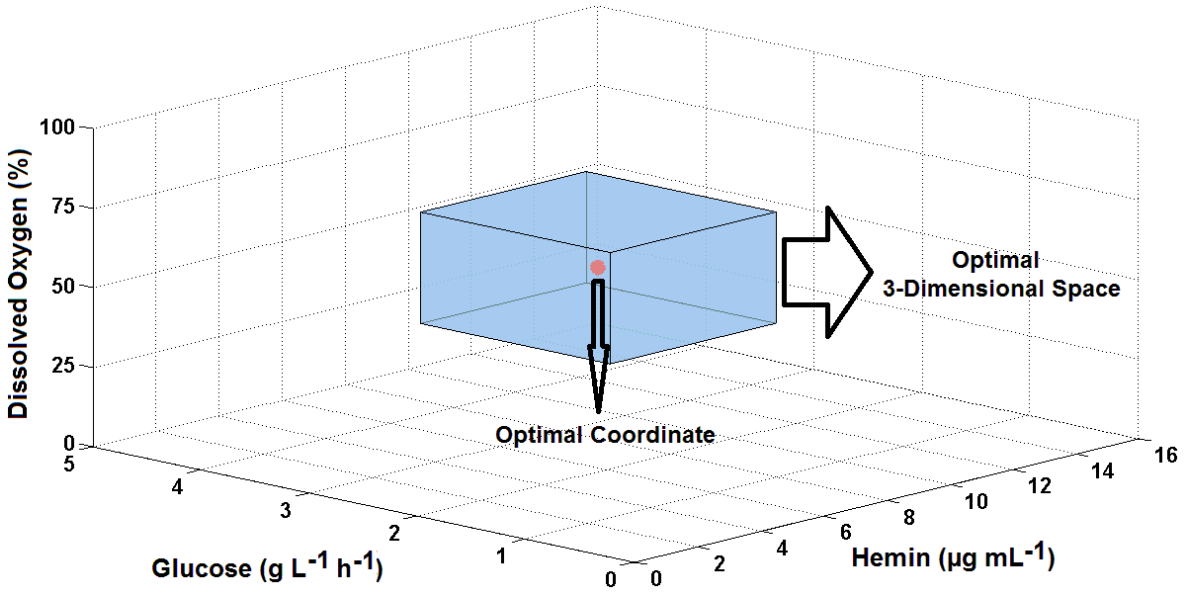

Figure 3. The tolerance limits of optimal variable values and the precise optimal coordinate that were created by decision trees to produce the optimal experimental results

These limits in Figure 3 are also related to the graphical results generated by modeling in Figure 2. In other words, the limits of optimal values created by decision trees are the same in both Figures 2 and 3. The Figure 2, Figure 3 and Table 2 show that the decision trees can very successfully determine the optimal variable values that produce the optimal experimental result because the optimal variable values determined by response surface method in literature are nearly same.

In the same optimization experiment in which the response surface analysis method was used in the previous study, it was determined that optimal values were 3 for glucose, 8 for hemin and 40 for dissolved oxygen. These previous study results are similar to the results of

this study while determined optimal oxygen values are slightly different. So the decision trees method can be reached to goal of the response surface methodology. However, decision tree method has some advantages as compared to response surface methodology. One of these advantages is that the decision trees method not only determines the optimal test result but provides the interval (Cyan cube in the Figure 3) of optimal experimental results also. This makes the optimal coordinate more sensitive to possible changes that may occur due to various factors.

Another advantage is that decision trees can determine the limits of variable values for all experimental results. So that in experiments if there is two optimal experimental results the optimal variable value ranges can be determined for both coordinates. Table 3 shows the optimal range of variable values for all experimental result limits determined by decision trees method.

Table 3. Optimum limits of variable values to be selected to produce the all experimental result ranges

Nisin (IU mg^{-1})	Glucose (g L^{-1} h^{-1})	Hemin (µg mL^{-1})	Dissolved Oxygen (%)
For Nisin<500	" > 14.65" *and* " < 15"	" > 4.4" *and* " < 4.5"	" > 77.3" *and* " < 80"
For 1000>Nisin>500	" > 13.53" *and* " < 14.65"	> 0.5 *and* <1.32" *or* " > 4.12 *and* < 4.4"	" > 69.5" *and* " < 77.3"
For 1500>Nisin>1000	" > 1" *and* "<5.83" *or* " > 11.57" *and* " < 13.53"	" > 1.32 *and* <2.36" *or* " > 3.64 *and* < 4.12"	" > 20" *and* "<31.1" *or* " > 56.3" *and* " < 69.5"
For Nisin>1500	">5.83" *and* " < 11.57"	" > 2.36" *and* " < 3.64"	" > 31.1" *and* " < 56.3"

As seen in the Table 3, decision trees method can calculate limits for the experimental design and determine relation between the experimental results and these limits. If desired, more precise limits can be specified by reducing the ranges of experimental results.

Furthermore, the decision trees method is compatible with all modeling algorithms. Even, it can also achieve the goal using the raw experimental data without modeling. In this case, the decision trees method eliminates one of the response surface methodology disadvantage arising from second degree polynomial dependency. In addition, the experimental design should be performed with a linear increase of the variable values so that the proposed method can determine the variable value intervals more efficiently. Because, if the variable values are not homogenous in the experimental design, the optimal variable values determine in very wide ranges and error possibility increase.

CONCLUSION

In this study, the use of decision tree method in food processing experiments has been proposed. The proposed method has been tested on a sample experiment the result is dependent on 3 variables. According to the findings obtained, the method has not only determined the variable values to produce the optimal experimental result but also determined the tolerance ranges of optimal variable values. Thus, the ranges have also been determined for experiments that the optimal variable values may possibly change depending on various factors. In addition, the proposed method can also determine the both optimal variable value ranges in cases where there is more than one optimal experimental result in the experiments. Furthermore, the proposed method can be used together with all modeling methods as well as being able to achieve its purpose without modeling also. As a result, it can be said that

proposed method can eliminate the many deficiencies of the response surface methodology which is the well-known and mostly used current optimization method of food processing experiments. Moreover, the proposed method aiming at optimization will be an alternative to the field of food engineering which is quite lacking in terms of optimization methods.

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The Optimization Method by Using the Transformation of Two Variable Dependent Experiment Results into Image Data and Its Usability in the Food Engineering Applications

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Abstract

In this study, it is aimed to determine the variable values which should be selected to produce the optimal experiment results in the field of Food Engineering by using image processing methods. In the study, the matrix of experiment results dependent on two variable values is transformed into the gray-scale image matrix, then the cells with the darkest color values (cells with black color is the least-valued) in the image matrix were identified. Finally, the variable limits (coordinate limits) of the black color cells have been determined. Determined limits were considered to be variable limits which will produce the optimal result of the experiment. The method proposed in the study has been tested in an exemplary experiment in which the antimicrobial effect of the *Lactobacillus casei* Shirota against the *Staphylococcus aureus* is determined by *in-vitro*. According to the obtained findings, it was confirmed that the proposed method can be used to determine the optimum variable limits in similar Food Engineering analyzes. Also which of the image processing methods would be useful in such optimizations were proposed.

Keywords: Antimicrobial effect, Image processing, Optimization, Two variable dependent analysis

INTRODUCTION

The Optimization is used in Food Engineering processes to analyze and determine the interactions of independent variables with each other and their effects on the target in order to produce the optimal results. The optimal result called as target function is the experimental result value desired to maximize or minimize with increasing or decreasing the independent variables according to predetermined criteria. In Food Engineering, response surface methodology that uses simple empirical models derived from trial sets is the most frequently used optimization methods. For example, in the analyses of hazelnut roasting processes, production of β-carotene and pulluan with using fermentation methods, protein recovery by using foam separation methods, determination of tarhana viscosity, determination of enzymatic browning of apple juice, inactivation of *Listeria innocua* etc., there are many successful results were offered to literature by using response surface methodology (Saklar et. al., 2001; Goksungur et al., 2004; Ibanoglu& Ainsworth, 2004; Ürküt et al., 2007; Aksay&Mazza, 2007; Buzrul, 2008).

Therefore, it can be said that the response surface methodology widely used in other engineering fields seems very useful in Food Engineering.

However, recently the new optimization methods through the help of computer softwares, mathematical and statistical methods, machine learning and data mining methods, etc. have been developed and are rapidly integrated into many engineering applications while Food Engineering has still lack of these developments.

The reason of this lack is the integration of newly developed optimization methods of Food Engineering are hard as compared to other engineering disciplines because food ingredients simulation and modeling is not easy due to the complexity of the physicochemical characterization. Also, it can be said that the response surface methodology is an only one and unique optimization technique used in the field of food science and technology because of this complexity problem for now. However, the response surface method has some problems of polynomial grade selection, experience requirement for initial trials, etc. (Koç&Kaymak-Ertekin, 2010).

So, the integration of optimization methods to Food Engineering is an important necessity to increase method varieties and to overcome disadvantages of old current methods. For example, the image processing techniques can be suggested as a solution because the image processing applications have begun to be used recently in food industry and effective agriculture (Kılıç et al., 2006; Samtaş&Gülesin, 2012; Sofu et al., 2013). Some researchers have performed the determination of the dimensional properties of the selected food samples, the classification of the samples in quality and color, the analysis of the gel image and the examination of the microscope images of the proteins etc. by using image processing technique. However, to analyze the experimental results by converting it into image data has not yet been attempted.In fact, all images consist of image values in the cells of an image matrix. Therefore, image processing techniques can be applied to all data including experimental results.

In this study, the original data matrix consisting of the experimental results was transformed into the image matrix and the limits of the independent variables which will produce optimal results by using image processing techniques are determined. Then the offered method was tested on a sample experiment related with antimicrobial effect of *L. casei* Shirota against *S. aureus* by *in-vitro*. As a result of the test, a new optimization method which can be used in the field of Food Engineering was proposed.

MATERIAL and METHOD

Material

A sample experiment was used to test the proposed method. In the sample experiment, antimicrobial effect of *Lactobacillus casei* Shirota which is one of the probiotic lactic acid bacteria exhibiting antimicrobial activity against *Staphylococcus aureus,* which is an important food pathogen was measured in terms of time and concentration variables by *in-vitro*. In the experiment, *S. aureus* was grown on Nutrient Broth (Merck) medium at 37 °C for 18-24 h, with a concentration of 10^5-10^6 / mL. *L. casei* Shirota was incubated for 24 h at 30 °C on MRS Broth (Merck) medium and with a concentration of 10^6 / mL. For determination of antimicrobial activity, 50, 100, 150 and 200 µL of *L. casei* Shirota was added onto *S. aureus* which in liquid medium (Nutrient Broth, Merck).

Samples which added *L. casei* Shirota were incubated at 37 °C and *S. aureus* colonies were counted after incubation for 0, 4, 8, 12, 24, 36, 48, 60 and 72 hours at Baird-Parker Agar (Merck) medium. In addition, the analysis was made in 3 replications for each time value. As

a result, the 27×4 dimensional original data matrix was created. In this original data matrix the lowest microorganism value is the optimal result (output) measured by analyses and the variable values (inputs) that will produce this optimal output are the optimal input values. The original data matrix is shown in Table 1. Matrix cell values are the antimicrobial effect of *L. casei* Shirota against *S. aureus* as microorganism concentrations. *S. aureus* concentration unit was given as log cob/mL.

Table 1. The experimental results matrix (original matrix) used to test the proposed method

	Concentration of *Lactobacillus casei* Shirota (log cob/µL)			
Time(hours)	**25**	**50**	**100**	**150**
0	6.32	6.25	6.17	6.02
0	6.28	6.18	6.02	5.96
0	6.31	6.22	6.13	5.88
4	5.87	5.86	5.67	5.60
4	5.80	5.92	5.53	5.47
4	5.63	5.42	5.58	5.63
8	6.21	6.15	6.05	6.00
8	6.32	6.23	6.12	6.02
8	6.12	6.08	5.95	5.96
12	6.88	6.85	6.81	6.79
12	6.75	6.89	6.75	6.66
12	6.96	6.79	6.88	6.89
24	7.92	7.90	7.80	7.74
24	7.89	7.74	7.75	7.68
24	7.96	7.98	7.91	7.82
36	7.65	7.54	7.12	7.08
36	7.58	7.65	7.08	7.11
36	7.63	7.49	7.22	7.09
48	7.72	7.43	7.31	7.15
48	7.63	7.56	7.22	7.08
48	7.8	7.37	7.45	7.19
60	7.81	7.62	7.25	7.21
60	7.88	7.58	7.36	7.36
60	7.75	7.70	7.14	7.07
72	7.58	7.83	7.47	7.37
72	7.45	7.93	7.56	7.24
72	7.69	7.69	7.33	7.49

Transforming data to image

The gray scale image is a matrix containing 256 color values from 0 to 255 in each cell (pixel). As these values increased the resulting color tone will change from black to white (0 means black, 255 means white and between 0-255 means gray tones). If the image is colored, there are three matrices containing the red, green and blue instead of gray tones.

The image depends on the colors that are created by superimposing (joining) these three matrices. The name of the 3D matrix formed by combining 3 matrices is the RGB matrix. Although color images are useful for visual analysis, grayscale images are more useful for analysis (Russ, 2016). The grayscale image sample and its matrix can be shown in Figure 1.

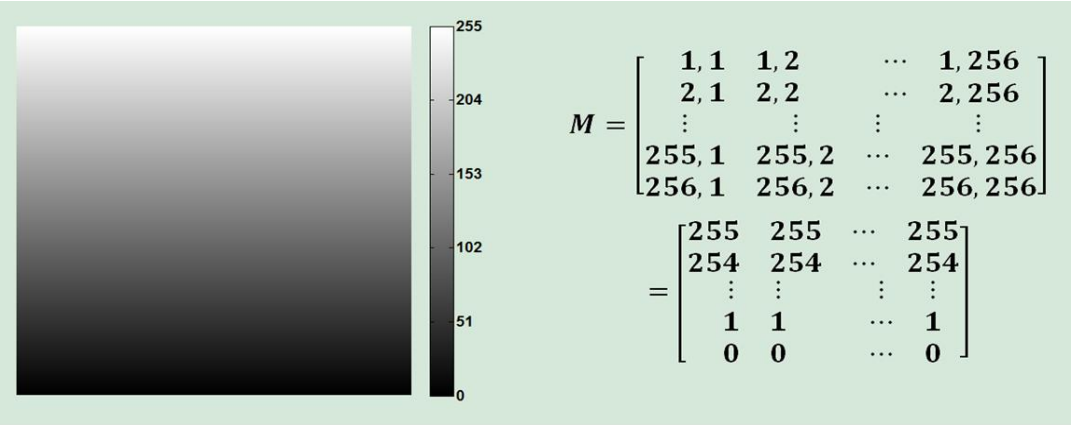

Figure 1. A grayscale, 256 x 256 resolution (256 x 256 pixels) sample image and its matrix

In this study, the method proposed as transforming experiment result matrix (original data matrix) into image matrix. Transformation of data matrix to image matrix can be done by equalizing (normalizing) the cell values to between 0 and 255 (Burger&Burge, 2016). Digital image processing: an algorithmic introduction using Java. Springer. Matrix normalization method for image creation is shown in Equation 1.

$$\mathbf{M} = \left\| \left(\mathrm{D} - \mathrm{D}_{\min} \right) \times 255 \right) / \left(\mathrm{D}_{\max} - \mathrm{D}_{\min} \right) \| \qquad (1)$$

In Equation 1, D is the data matrix, $\mathrm{D}_{\min}$ is the minimum value of D, $\mathrm{D}_{\max}$ is the maximum value of D and M is the image matrix.

Increasing Image Resolution

The larger the size of an image matrix (the higher the resolution) means the better its visual analysis. At the same time, the greater resolution means the more precisely the coordinates of the optimum variable values of the data matrix can be determined. There are two ways to increase the image resolution. The first is the addition of new cells around each cell in the image matrix. In this first method, the values of cells to be newly created, are calculated by the averages of the values in neighboring cells. However, since artificial colors are added with this method, the image will be a bit different from the original and the resolution will also deteriorate (Lau & Lin, 2016). The second method is to increase the size of the original data matrix. This is can be done by a polynomial model that produces output values corresponding to the variable values. Namely, the larger data matrix can be obtained by calculating the output values (experiment result values) corresponding to the unused variables in the model polynomial. As a result, the size of the image matrix will be larger and the resolution will be higher. In this study, the polynomial model was constructed using the Vandermonde matrix.

Reason of this method use is the product of the Vandermonde matrix with the polynomial coefficients is the outputs (Experiment result values) corresponding to the variable inputs (Cordova et al., 2016). Using the Vandermonde matrix the polynomial coefficients and model polynomials can be determined as follows.

Assume that $\mathrm{x}_{\mathrm{n,m}}$ is the m^{th} value of n^{th} input variable and y_{n} is the output value corresponding to input variable $\mathrm{x}_{\mathrm{n,m}}$. In this case the relation between the input variable $\mathrm{x}_{\mathrm{n,m}}$ and output value y_{n} is as in Equation 2. So, the polynomial model is Equation 2.

$$c_{n,m}{x_{n,m}}^{n} + \cdots + c_{2,m}{x_{n,m}}^{2} + c_{1,m}{x_{n,m}}^{1} + c_{0,m}{x_{n,m}}^{0} = y_{n,m} \quad (2)$$

The c_n values in Equation 2 are polynomial coefficients can be calculated as in equation 3.

$$\begin{bmatrix} {x_{0,m}}^{0} & {x_{0,m}}^{1} & {x_{0,m}}^{2} & \cdots & {x_{0,m}}^{n} \\ {x_{1,m}}^{0} & {x_{1,m}}^{1} & {x_{1,m}}^{2} & \cdots & {x_{1,m}}^{n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ {x_{n,m}}^{0} & {x_{n,m}}^{1} & {x_{n,m}}^{2} & \cdots & {x_{n,m}}^{n} \end{bmatrix} \begin{bmatrix} c_{0,m} \\ c_{1,m} \\ \vdots \\ c_{n,m} \end{bmatrix} = \begin{bmatrix} y_{0,m} \\ y_{1,m} \\ \vdots \\ y_{n,m} \end{bmatrix} \quad (3)$$

Since there are two variables in this study, the m value in Equation 2 and Equation 3 was assigned as first "1" then "2". Thus, polynomials were created as $y_{n,m} = y_{n,1}$and $y_{n,m} = y_{n,2}$.Since $y_{n,1} = y_{n,2}$ for the same n value, the polynomial with two variables was also calculated as $P_{a,b} = \frac{y_{a,1}+y_{b,2}}{2}$ for $a = \{1,2,\dots,n\}$ and $b = \{1,2,\dots,n\}$. However, note that the other methods can be used to create polynomial models instead of providing method based on vandermonde matrix.

RESULTS and DISCUSSIONS

In an experiment, the smallest or largest value of the resulting value from the input variable is the optimum result value. Therefore, the minimum (black color with a value of 0) or maximum (White color with a value of 0) values are the optimums of outputs in the image matrix. In the image matrix, the row and column coordinates of these values are the variable values that will produce the optimal result also. The sample data matrix used in the study is the data matrix created to measure the antimicrobial effects of *L. casei* Shirota that is a probiotic lactic acid bacterium on *S. aureus*. The input variables of this matrix are time and *L. casei* Shirota concentration. The original data matrix is 27×4 dimensional and is modeled as 721×1521 dimensional by using polynomials. Thus, the dimension (resolution) of the image matrix was increased also. The visual result (image) of transforming the data matrix obtained by the polynomial model into the image matrix by normalization method is as shown in Figure 2.

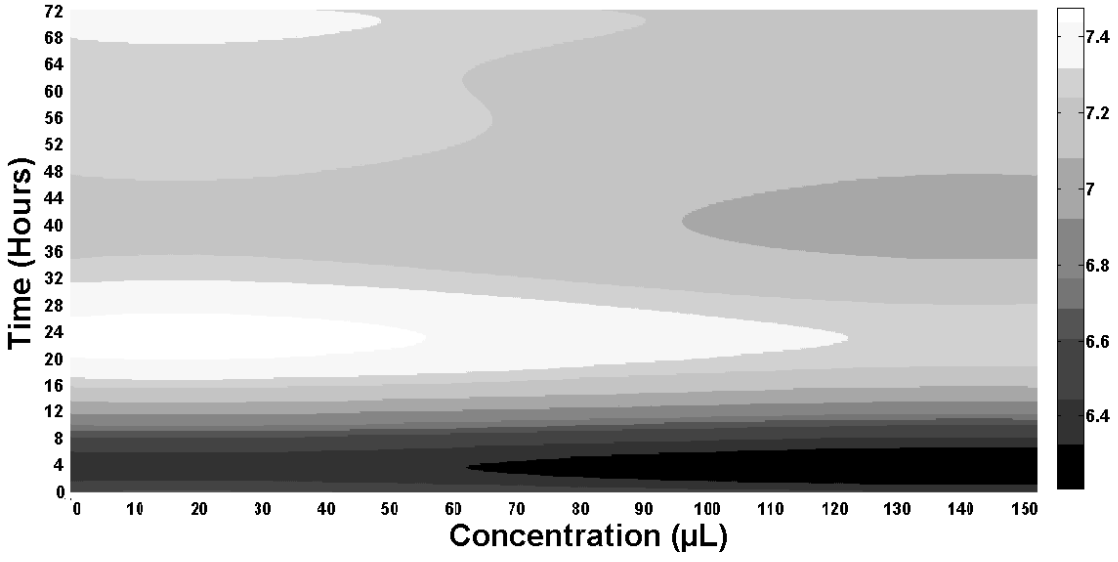

Figure 2. Image resulting from transforming of the original data matrix to image matrix

In Figure 2, the black colors are the cells with the lowest concentration of microorganism (The cells with optimum output values). If Figure 2 is analyzed visually it can be observed that the time variable that will produce the optimal outputs (optimal experimental results) are within 2-6 hours. According to the same visual analysis the input variable related with concentration to produce the optimal output is about within 60-150 µL range. However, although the human eye can distinguish up to 256 tones per color the non-adjacent contrast colors cannot be distinguished for more than 16 tones. In other words, the dark gray tones similar to darkest black color in the same region at Figure 2 may be thought to be the same tones as the darkest black color. Therefore, visual analysis of images can be deceptive and quantitative analysis of the image matrix will be more accurate. If the black cell coordinates of image matrix are calculated as in Equation 4 variable coordinates that will produce the

$$\boldsymbol{Find}\ D_x\ and\ \ D_y\ If\ D < 1 \tag{4}$$

optimal results can be determined precisely.

This method in Equation 4 is actually a low pass filtering for the image. According to result of filtering the value ranges of the input variables that will produce the optimal outputs are within 3.3-3.9 hours for the time and 131-149 µL for the concentration. The image obtained according to Equation 4 is also as shown in Figure 3. This is the filtered image of the region where the optimal outputs will be obtained (image of only the darkest black color cells).

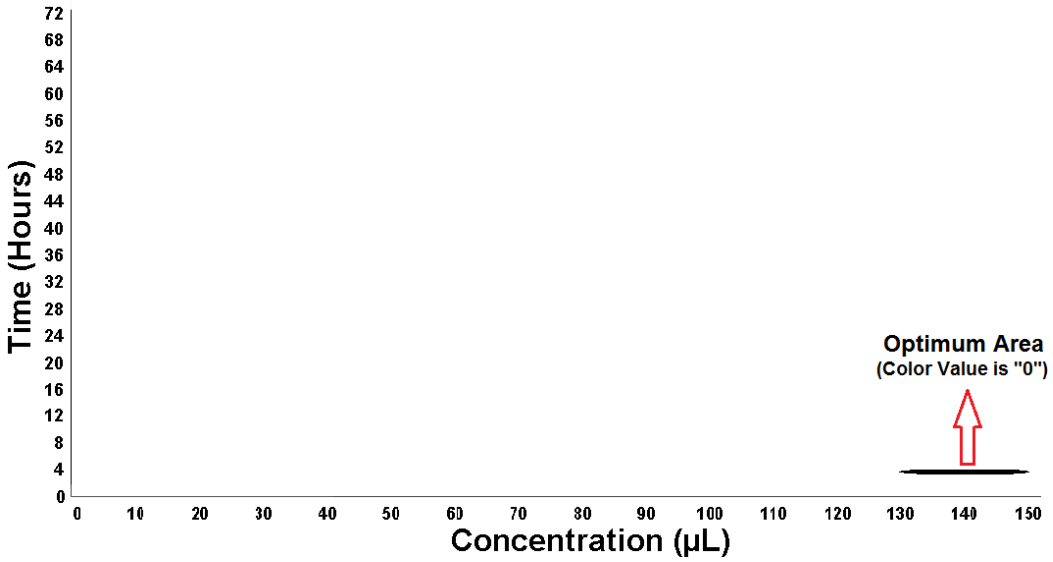

Figure 3. The Image of the region where optimum output is obtained by image filtering

By visually inspecting the image in Figure 3, the limits for optimum values of input variables can be more clearly determined. Thus, by applying low pass filtering for the image the limits of the input variable values which will produce optimum output can be observed more clearly. Image enlargement is another image processing technique can also be used for a clearer visual analysis. The image obtained by the image enlargement technique is as shown in Figure 4.

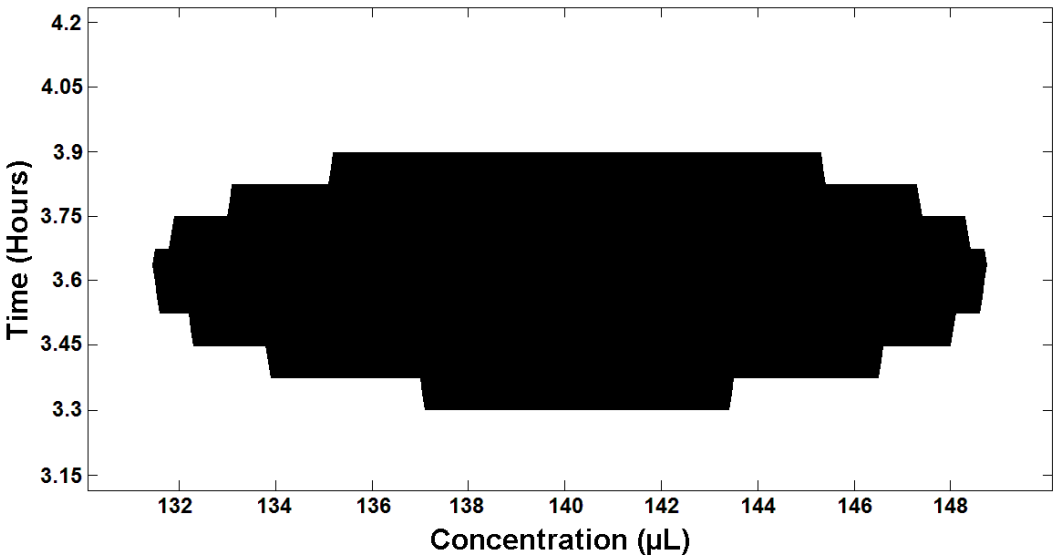

Figure 4. Enlarged image of the zone that will produce optimal output

Determined according to results from figure the value ranges of the input variables that will produce the optimal outputs are within 3.3-3.9 hours for the time and 131-149 µL for the concentration. So, the findings in equation 4 can also be visually observed.

In some cases, although the same input variable values are used, different output values can be generated due to many factors. In such cases it may be more accurate to determine the broader range for the optimized output. The suggested method is also suitable for such cases. Because instead of filtering only the minimum values of the image, the first 2 or 3 values closest to minimum can be also filtered. That is, by changing the threshold coefficient of the low-pass filter, a larger optimum region can be determined. If the optimum output is the maximum value resulting from the experiment then the highest values of the image can be selected by using high pass filter.

It is possible to determine the lowest output value in the original data matrix and identify the corresponding input variable values. However, it is not possible to determine the optimal output range. This is because the optimal output values can only be determined by visually or using data mining techniques. If so, the proposed method can be said to be more advantageous than the determination of the optimal output in the raw data matrix.

CONCLUSION

In this study, a new method proposed for the determination of the range of input variable values that should be used in order to produce optimal output. The proposed method is to create the modeled data matrix by using model related to input and output interaction of the experiment then transform the data matrix into the image matrix. By using this proposed method, it is possible to determine the ranges of variable values which should be used for the optimal experiment result to be produced. In food engineering applications, it is possible to determine the optimal independent variable values by means of some methods in the literature. However, these methods in the literature are not sensitive to the optimum output value which can vary with various factors. The proposed method is sensitive to change as it is in data mining methods. So, it is also possible to develop the method.

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