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ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER   **TAM MAKALE**

HEPATOPROTECTIVE EFFECT OF TOFU PROCESSED FROM GERMINATED SOYBEAN ON CARBON TETRACHLORIDE INDUCED CHRONIC LIVER INJURY IN MICE

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Abstract:

The hepatoprotective activities of silk tofu made from germinated and non germinated soybeans at different doses of feeding against CCl_4 induced hepatic cell toxicity in mice was investigated in this study. The hepatoprotective activity was analyzed by assessing the ratio of liver weight to body weight (L/B), the levels of serum alanine aminotransferase (ALT), total cholesterols (TC), the hepatic malondehydyde (MDA), protein carbonyl (PC) and vitamin C levels as well as the histopathological analysis of liver tissue. All types of silk tofu significantly reduced the L/B value; ALT activity, total cholesterol, hepatic MDA and PC levels, beside, liver vitamin C content increased compared to CCl_4 intoxicated mice. Silk tofu made from germinated soybeans expressed higher hepatoprotective activity as compared to silk tofu made from non germinated soybeans. Mice fed with silk tofu made from germinated soybeans at the dose of 0.4 g/g body weight/day displayed all biochemical parameters as well as the liver tissue histopathological analysis that were similar to that of normal mice and silymarin treated mice. It was suggested that tofu specially made from germinated soybeans expressed great hepatoprotective effect.

Keywords: Liver injury, Carbon tetrachloride, Silk tofu, Germination, Antioxidants

Introduction

Chronic liver dysfunction or injury is one of the most serious health problems and be considered the major cause of human mortality in the world (Wood, 2010; Abdel-Wahhab *et al.*, 2011). Chronic liver diseases were described clinically through pathological processes of the liver, involving a process of progressive destruction and regeneration of the liver parenchyma. Finally, if left untreated, these processes will lead to cirrhosis and hepatocellular carcinoma (Hong *et al.*, 2015).

Generally, liver injury is considered a result of exposure to different environmental pollutants and xenobiotics e.g., thioacetamide, paracetamol, carbon tetrachloride, alcohol, etc. (Lazerow *et al.*, 2005; Ashraf *et al.*, 2012). These xenobiotic compounds mainly damage liver by producing the reactive oxygen species (ROS) that induce the toxicity by covalent binding and lipid peroxidation (Geesin *et al.*, 1990). Among these chemical hepatotoxins, CCl_4 had been frequently used to induces toxicity in rat liver which closely resembles human cirrhosis. It produces reactive free radicals trichloromethyl radical (CCl_3) and a proxy trichloromethyl radical (CCl_3O_2) when metabolized (Yang *et al.*, 2015). CCl_4 causes hepatocyte injury that is characterized by centrilobular necrosis that is followed by hepatic fibrosis (Yu *et al.*, 2002). Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues (Thresiamma and Kuttan, 1996). Polyphenolic compounds from food materials are known to be excellent antioxidants in vitro because of the capacity to scavenge free radicals and protect antioxidant defense (Latha *et al.*, 1999). Beside, it is preferable due to lack of serious adverse effects.

Tofu is a phenolic rich soybean product accepted for consumption worldwide, mostly in Asian countries (Wu *et al.*, 2004). Tofu is rich in protein and a good source of vitamins, minerals, as well as antioxidants such as polyphenols, isoflavones, vitamins C and vitamin E (Poysa and Woodrow, 2002). It was also demonstrated to prevent acetaminophen-induced liver damage in rats (Yakubu *et al.*, 2013). A simple, efficiency and unexpensive process to enhance important antioxidants such as polyphenols, isoflavones, vitamin C and vitamin E in soybean is germination (Kaushik *et al.*, 2010; Paucar-Menacho *et al.*, 2010). Processing tofu from germinated soybean should be an effective mean to enhance the antioxidant compounds in the product that have a beneficiary effect to consumers. To demonstrate this, the protective effect of tofu produced from germinated soybean on the CCl_4 induced chronic liver damage in mice is investigated.

Materials and Methods

Germination of soybean seeds

Soybeans (*Glycine max* L., MTĐ 760 variety) were supplied from Department of Agricultural Genetic, College of Agricultural and Applied Biology, Cantho University.

Soybeans were cleaned and rinsed three times with cleaned water before being soaked for 12 hours at ambient temperature. The soaked beans were drained, rinsed and placed in a germination cabinet, which watered the seeds every 4 hours with cleaned water automatically, the time for watering was two minutes. The germination process was carried out at 25°C in dark condition for 42 hours.

Silk tofu preparation

Briefly, the germinated and non–germinated soybeans were rinsed and ground with hot water (water/dry weight of bean was 6/L, v/w) (Ndatsu and Olekan, 2012) by the crushing machine, the slurry was filtered through a three layers cheese cloth to obtain soy milk. Soy milk was boiled for 5 minutes and then cooled down 20°C. GDL (Glucono-delta-lacton) 3g/L was added and mixed well. The soymilk was then filled to boxes, sealing them and they were immersed in water bath at 90°C and 44 minutes for coagulation. The silk tofu products were stored at ≤ 5°C for 1 day to analyse the total polyphenol content (TPC) antioxidant activities.

Determination of TPC and antioxidant activity of silk tofu

Tofu samples were freeze dried to fine powder before analysing. The extraction procedure for analysing was carried out by method of Duong *et al.* (2015).

Determination of the TPC

The TPC of tofus were estimated by Folin-Ciocalteu method (Jiang *et al.*, 2013). The total phenolic content of samples was expressed as milligrams garlic acid equivalents per gram of dry matter (mg GAE/g).

Determination of antioxidant activity

Antioxidant activity of silk tofu extracts were assessed by measuring their scavenging activity of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. This procedure was described by Liu *et al.* (2011). Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract (μg/mL) to obtain IC50 value in mg/mL. The results were showed in Table 1.

Animals

Male white mice (*Swiss albino* strain) were obtained from the Pasteur Institute, Ho Chi Minh city, Vietnam. They were 5 to 6 weeks old (25–30g) and were allowed free access to pellet diet and water ad libitum to acclimatize for a week prior to experimentation. Mice were housed in plastic mesh cages in the laboratory of Department of Pharmacology, Cantho University of Medicine and Pharmacy, under ambient temperature and 12 h light and dark cycle.

Experimental design

Forty-two mice were divided into seven groups (each group consisted 6 mice).

Group (1): Normal control group, animals were treated with olive oil (10mL/kg b.w., o.p. three days for once).

All other groups, mice were treated with 10mL (CCl_4 20% in oliu oil)/kg b.w., o.p. three days for once. In addition, they would be treated simultaneously in different ways, as followings:

Group (2):   Control positive group (mice were treated with CCl_4 only).

Group (3):   Control negative group, mice treated oral doses of 16mg silymarin/kg b.w. one hour after CCl_4 toxicititation.

Group (4) and (5): Mice were fed with silk tofu 0.2g/g b.w./day (ST low) and 0.4g/g b.w./day (ST high) respectively.

Group (6) and (7): Mice were fed with silk tofu made from germinated soybean 0.2g/g b.w./day (GST low) and 0.4g/g b.w./day (GST high) respectively.

The experiment was carried out during 6 weeks. At the end of the experiments, blood and livers were collected immediately after the animals were sacrificed. Blood was determined the ALT and TC in serum. The liver from each animal was determined the L/B, PC, MDA, vitamin C contents and histology properties.

Determination of serum ALT and TC, liver PC, MDA, vitamin C contents and histology properties

Determination of serum ALT, TC and liver histology property

Blood and liver samples were sent to Cantho University Hospital for analysing of serum ALT and TC by ARCHITECT–Ci4100 machine (Abbott Company, America) and hepatic histology property. The degree of fibrosis was evaluated in the liver tissue according to the Hepatitis Activity Index (HAI) (Ishak *et al.*, 1995) which scores of fibrosis were based on Knodell – Ishak scales from 0 to 22.

Determination of liver PC

The PC values were measured by spectrophotometric method at the absorbance of 370 nm, using dinitro-phenylhydrazine (DNPH) reagent (Levine RL, 1990). Results were calculated as nanomoles of carbonyl groups per milligram of protein (nmol/mg protein). Total protein was determined by Bradford assay (Bradford, 1976) that relies on the binding of the dye Coomassie Blue G250 to protein that has an absorbance maximum at 590 nm. The quantity of protein can be estimated by determining the amount of dye in the blue ionic form by measuring the absorbance of the solution at 595 nm.

Determination of liver MDA

The MDA levels of liver tissue were carried out using the modified method of Ohkawa *et al.* (1979). MDA is a product of lipid peroxidation that reacts with acid thiobarbituric (TBA) under acidic conditions forming a pink complex that absorbs at 532 nm. Malonaldehyde bis (Acros–Belgium) was used as the standard. The results are expressed as nmol/mg protein.

Determination of the liver vitamin C content

Vitamin C contents in liver tissue were determined by the spectrophotometric method of George (2003) that is based on the reaction with 2,4-dinitrophenylhydrazine reagent. The optimum absorbance of reaction product color was 520 nm. A standard was prepared using of pure ascorbic acid. The results are expressed as μg/mg protein.

Statistical analysis

The data were submitted to analysis of variance (ANOVA) by Portable Statgraphics Centurion 15.2.11.0 and were expressed as mean values and standard deviation.

Results and Discussion

The L/B, serum ALT and TC values from seven experimental mice groups were presented in Table 2. The MDA, PC and vitamin C contents in mice liver tissues from these groups were showed in Table 3. Histological examination of mice liver tissues was displayed in Figure 1.

The L/B ratio were increased 60% in mice treated with CCl_4 (Control positive group) as compared to that of control mice. Feeding mice with silk tofu (ST low, ST high, GST low and GST high) reduced the L/B values to 8.3; 10.0; 13.6 and 23.7% respectively. In which, the L/B values of mice from ST high, GST low and GST high groups similar to L/B value of mice treated with silymarin (Control negative group), whose L/B value was remained closing to L/B value of normal group (Table 2).

Serum ALT increased 344% in mice treated with CCl_4 comparing to ALT of control mice. ALT value of mice treated with silk tofu (ST low, ST high, GST low and GST high) restricted the increase in serum ALT (the decreasing of 46.7, 61.7, 61.0 and 70.9% respectively) as compared to that of mice treated with CCl_4. Within them, tofu made from germinated soybeans (GST low and GST high) showed the higher effective in the ALT restoration. Specially, the ALT value in mice fed with high dose (0.4g/g b.w./day) of silk tofu made from germinated soybeans was similar to that of normal control group and control negative group (Table 2).

A significant increase in serum TC levels (43.9%) were observed in CCl_4 treated mice, compared to the control group. Four groups of mice fed with silk tofu attenuated the increased levels of serum TC that resulted from the treatment previously with CCl_4. The TC value from mice group fed with high dose of silk tofu made from germinated soybeans was not significant different with TC values from normal control group and control negative group (Table 2).

In this study, CCl_4 treatment markedly increased (50.2%) the hepatic MDA level as compared with the normal control group. Treatment with silk tofus significantly reversed this change. MDA levels in mice from ST low, ST high, GST low and GST high groups reduced 9.6, 16.0, 15.9 and 23.5% respectively as compared to hepatic MDA level of control positive group. The MDA value from mice group fed with high dose of silk tofu made from germinated soybeans was not significant different with MDA value from control negative group (Table 2).

The present study detected a significant increasing (64.9%) in liver PC content of the CCl_4 treated mice as compared to control mice. The PC levels in four mice groups fed with tofu decreased significantly when compared with that of control positive group. Tofu made from germinated soybeans also displayed as the more effective agents in the reversion of the change in PC content caused by CCl_4 toxication.

The level of vitamin C in liver of CCl_4 control group significantly decreased in comparison with the normal control group (54.6%). After application of silymarin as well as silk tofu as ST low, ST high, GST low and GST high groups the increase the levels of hepatic vitamin C by 101.0, 49.5, 74.9, 73.8 and 95.9% respectively, as compared to that of CCl_4 treatment group.

The results of liver histopathology from Figure 1 of seven *Swiss albino* mice groups were described more detailed in Table 4.

Table 1. The TPC and IC50 values of silk tofus made from germinated and non–germinated soybeans

	Silk tofu (Germinated soybeans)	Silk tofu (Non–germinated soybeans)
TPC (mg GAE/g d.w.)	3.39[b]±0.03	2.45[a]±0.09
IC50 (mg d.w./mL)	14.09[a]±0.12	15.37[b]±0.14

(Means ±SD, the values showing different superscripts within a row are significant different at P<0.05)

Table 2. The L/B, serum ALT and TC values of experimental mice groups

Groups	L/B (%)	ALT (U/L)	TC (mg/dL)
1. Normal control group	$3.30^a\pm0.20$	$48.83^a\pm0.02$	$105.51^a\pm12.61$
2. Control positive group	$5.28^e\pm0.15$	$216.83^d\pm37.94$	$151.83^d\pm32.63$
3. Control negative group	$4.25^{bc}\pm0.47$	$58.83^a\pm8.16$	$106.79^{ab}\pm7.97$
4. ST low group	$4.84^{de}\pm0.49$	$115.67^c\pm18.89$	$130.60^c\pm8.94$
5. ST high group	$4.75^{cd}\pm0.44$	$83.00^b\pm4.00$	$120.95^{bc}\pm3.15$
6. GST low group	$4.56^{cd}\pm0.71$	$84.50^b\pm3.89$	$120.95^{bc}\pm3.15$
7. GST high group	$4.03^b\pm0.35$	$63.17^a\pm7.41$	$104.22^a\pm4.23$

(Mean s±SD, the values showing different superscripts within a column are significant different at P<0.05)

Table 3. The liver tissue MDA, PC and vitamin C values of experimental mice groups

Groups	MDA (nmol/mg protein)	PC (nmol/mg protein)	Vitamin C (µg/mg protein)
1. Normal control group	$7.77^a\pm0.65$	$5.04^a\pm0.18$	$10.69^e\pm0.81$
2. Control positive group	$11.67^e\pm0.30$	$8.31^e\pm0.30$	$4.85^a\pm0.47$
3. Control negative group	$8.58^b\pm0.42$	$5.44^b\pm0.44$	$9.75^d\pm0.83$
4. ST low group	$10.55^d\pm0.52$	$7.38^d\pm0.39$	$7.25^b\pm0.18$
5. ST high group	$9.80^c\pm0.17$	$6.75^c\pm0.25$	$8.48^c\pm0.41$
6. GST low group	$9.81^c\pm0.38$	$6.50^c\pm0.22$	$8.43^c\pm0.33$
7. GST high group	$8.93^b\pm0.38$	$5.22^b\pm0.24$	$9.50^b\pm0.47$

(Means ±SD, the values showing different superscripts within a column are significant different at P<0.05)

Table 4. Liver histopathology description and chronic hepatitis degrees of *Swiss albino* mice from seven experimental groups

Groups	Descriptions	Scores (HAI)	Degrees of chronic hepatitis
(1)	Liver tissues presented with normal histological structure, hepatocytes and venous sinusoids are arranged as interconnected plates (Figure 1.A)	0	No inflammation
(2)	Appearing many inflammatory cells as well as necrotic cells in the lobules, widening of portal area, the disarrangement of hepatocytes and venous sinusoids around the central lobules at serious level (Figure 1.B).	10	Moderate chronic hepatitis
(3)	Necrotic cells could not be found in lobules, but there was very little inflammatory and necrotic cells at portal area (Figure 1.C).	3	Very mild chronic hepatitis
(4)	There was little inflammatory and necrotic cells in lobules and portal area (Figure 1.D).	4	Mild chronic hepatitis
(5)	Moderate appearance of necrosis in lobules and portal area, there was little inflammatory cells at portal area (Figure 1.E).	8	Mild chronic hepatitis
(6)	Necrotic cells could not be found in lobules, but there was very little inflammatory and necrotic cells at portal area (Figure 1.F).	3	Very mild chronic hepatitis
(7)	There was little inflammatory and necrotic cells in lobules and portal area (Figure 1.G).	4	Mild chronic hepatitis

CCl_4 is a well known hepatotoxic agent and the most remarkable pathological characteristics of CCl_4 induced hepatotoxicity are fatty liver, cirrhosis and necrosis (Huo *et al.*, 2011). It could result in an increasing of blood content, to the dilatation of central veins and sinusoids, swelling of hepatocytes resulted from the increase in water transport in cells and fatty liver or due to the increase in accumulation of fat in hepatocytes. All of these reasons could lead to increase in L/B of CCl_4 treated mice (Robins *et al.*, 1979; Huo *et al.*, 2011). Increasing in L/B coincides with many previous results from studying of hepatotoxicity on mice by CCl_4 (Domitrović *et al.*, 2009; Huo *et al.*, 2011).

It is well documented that CCl_4 enhanced lipid peroxidation (Abdel-Wahhab *et al.*, 2006; El Denshary *et al.*, 2012). The CCl_4 induces the peroxidation of lipids that damage the membranes of liver cells and organelles. This results in the release of ALT that is found outside of the mitochondria of the liver into the circulating blood (Shankar *et al.*, 2008) leading to increasing the levels of liver enzymes (ALT). The rising in ALT activity is almost always due to hepatocellular damage (Ravikumar *et al.*, 2005). Essawy *et al.* (2012) reported that serum ALT of *Swiss albino* mice treated with CCl_4 at a dose level 1.9 mL/kg b.w increased 328.8% when compared with ALT value of control mice.

Distinct alterations in lipid metabolism have been reported in CCl_4 induced hepatotoxicity in rats (Singhal and Gupta, 2012). The liver is the major site for the synthesis and metabolism of cholesterol (Yang *et al.*, 2011). CCl_4 increases the transport of acetate into the liver cell, resulting in increased acetate availability, for this reason, the cholesterol synthesis from acetate was also increased (Boll *et al.*, 2001). Sarhan *et al.* (2012) reported that TC levels in *Sprague Dawley* male rats much higher after the treatment with CCl_4 for 8 weeks.
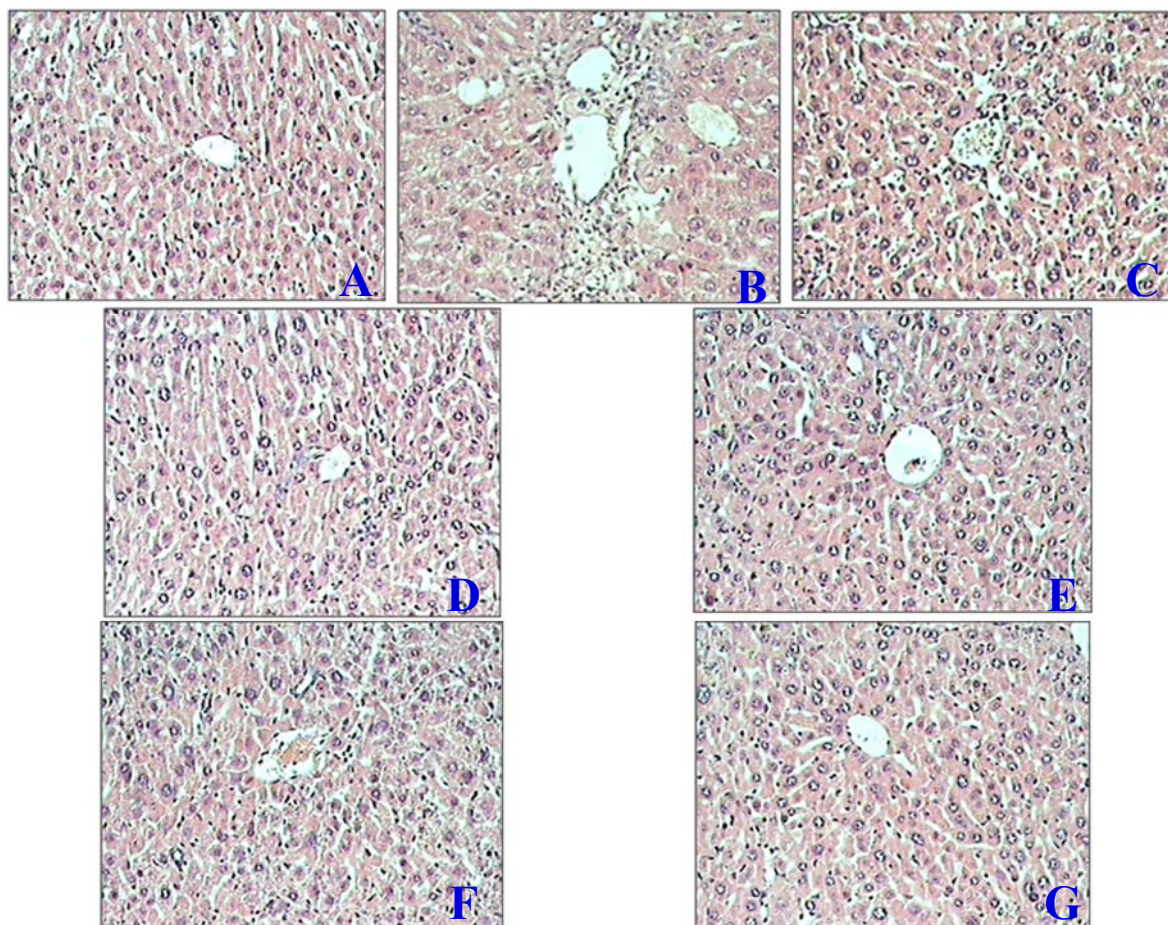
(H and E staining, magnification x 100)

Figure 1. Micrographs from representative liver tissues collected from mice from group (1) (Figure 1.A); group (2) (Figure 1.B), group (3) (Figure 1.C), group (4) (Figure 1.D), group (5) (Figure 1.E), group (6) (Figure 1.F) and group (7) (Figure 1.G).

The result of the peroxidation of lipids induced by CCl_4 is the formation of MDA and its level in liver tissue was assessed as an indicator of lipid peroxidation in oxidative liver damage (Nielsen *et al.*, 1997). The present results in liver MDA increasing of CCl_4 treated *Swiss albino* mice are consistent with previous study (Saad, 2013). Another aspect as regards to oxidation of proteins. Protein oxidation may play a role in the pathogenesis of CCl_4 induced liver injury (Sundari *et al.*, 1997) and the accumulation of oxidised proteins in the liver may be an early indication of CCl_4 liver injury. The PC that is product from the free radical-mediated oxidation of proteins (Robinson *et al.*, 1999), is widely used as a indicator for measuring of oxidative damage (Luo and Wehr, 2009). The advantage of using protein carbonyl as a marker may be due to the relatively early formation and stability of oxidized proteins (Dalle-Donne *et al.*, 2003). The result in the increase of hepatic PC due to CCl_4 treatment from

this study coincided with the results of Sundari *et al.* (1997) in the model of chronic rat liver injury.

In the present study, the decrease in the liver vitamin C level induced by CCl_4 indicated was detected. CCl_4 generated ROS causing the feed-back inhibition or oxidative inactivation of enzyme protein leading the decrease antioxidants (such as GSH) in plasma and tissue (Pigeolet *et al.*, 1990). This resulted subsequently in reduction of other antioxidants such as ascorbic acid and aggravate the cells to further damage (Al-Assaf, 2014).

The above changes related to CCl_4 induced liver injury expressed an indication of structural and functional defects in liver cells that was proved in histopathological examination (Figure 1.B and Table 4). It was clearly established that necrosis and inflammatory cells were observed in the liver sections of animals treated with CCl_4. These damages observed on the liver architecture were

expression of moderate chronic hepatitis and they might be associated with the production of oxidative stress caused by CCl_4 intoxication.

Feeding mice with various forms of silk tofus (ST low, ST high, GST low and GST high) had tendency to reduce L/B, serum ALT and TC as well as the hepatic MDA and PC values. However, the vitamin C content increased and finally, the liver injury was improved through the histopathological examination (Figure 1.A, C, D, E, F and G). This histopathological observation could be attributed to the potent antioxidant activities of tofu polyphenol compounds that are potent free radical scavengers in the body system. Tofu made from germinated soybeans showed greater effective than that of tofu made from ungerminated soybeans in protection against CCl_4 induced hepatic toxicity. Especially, feeding mice with tofu made from germinated soybeans at the dose of 0.4g/g b.w./day (GST high) remained the biochemical properties of mice liver as closing to that of mice from normal control group and mice treated with silymarin. Interestingly, intact hepatic cell architectures were observed in mice from this group and this normal histological structure was similar to liver cell sections of the normal control and control negative group (Figure 1.A, C and F).

Phenolic compounds in soybeans and soy products were natural antioxidants which functions as a potent neutralizer of free radical species in the body and they acted against the liver damaging effects of free radicals produced by CCl_4 (Yakubu *et al.*, 2013; Yakubu and Mohammed, 2016). Tejasari *et al.* (2014) proved through both the histopathologic observations and statistical analyses that the administration of soy extract can provide protection against mouse liver tissue damage where injury is induced by CCl_4. Beside, the authors stated that soys inhibit the initiation of both the extrinsic and intrinsic apoptotic processes in pathways in hepatocytes is what ultimately could play a role in improving survival in conditions in a state of liver injury (Tejasari *et al.*, 2014).

Germination involves physiological changes, synthesis and breakdown of macromolecules, improving the digestibility and nutritive value of soybeans (Fernandez-Orozco *et al.*, 2008). This process enhances levels of important antioxidants such as polyphenols, isoflavones, vitamin C and vitamin E as compared to ungerminated soybeans (Paucar-Menacho *et al.*, 2010). So, the potential of free radical scavenging of germinated soybeans as

well as products from them were increased. In this study, TPC content of silk tofu made from germinated soybeans was 1.38 folds (Table 1) higher than that of silk tofu made from non–germinated soybeans. So, IC50 value of silk tofu made from germinated soybeans was lower than IC50 value of silk tofu made from non–germinated soybeans (by 91.7%, Table 1). For this reason, silk tofu from germinated soybean showed the greater hepatoprotective effects as compared to that of silk tofu from non–germinated soybeans.

Conclusion

The present study demonstrated that all silk tofus exhibited hepatoprotective activity against CCl_4 intoxication in mice. The liver protection ability of silk tofu may be associated with their free radical scavenging and antioxidant capacities. Specially, silk tofu made from germinated soybeans may be more efficacious hepatopreventive agent. Therefore, supplementation of tofu as well as food products made from germinated soybeans in our diets can be highly recommended as it can be used as a functional food to prevent liver injury.

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REVIEW ARTICLE   **DERLEME MAKALESİ**

A RISING STAR PREBIOTIC DIETARY FIBER: INULIN AND RECENT APPLICATIONS IN MEAT PRODUCTS

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Abstract:

Inulin is a soluble dietary fiber extracted by a washing process mainly from chicory roots. In recent years, inulin has been mentioned as an ingredient having an important application potential in various areas such as chemical, food industry and pharmacy. Since there has been a rising demand for consumption of healthier meat products all over the world due to high and saturated fat content of these products, it is important to suggest healthier ingredients that have an ability to compensate for fat replacement. There has been a growing increase in number of studies on the incorporation of inulin in the formulation of various meat products, due to the positive impacts of inulin on textural, sensory and technological quality parameters compared to full-fat products, as well as it has beneficial effects promoting human health. In this review, we have chosen to briefly highlight inulin in terms of its physico-chemical properties, health implications and potential applications in meat products.

Keywords: Inulin, Dietary fiber, Prebiotics, Healthier meat products

Introduction

Inulin is a natural storage polysaccharide of various plants which are mostly part of the *Compositae* family including chicory, dahlia, and Jerusalem artichoke. Inulin can also be produced by microorganisms including *Streptococcus* and *Aspergillus* species (Barclay et al., 2010; Glibowski and Bukowska, 2011). Other natural sources of inulin are yacon, asparagus, leek, onion, banana, wheat and garlic (Shoaib et al., 2016). Among these sources, in industrial production of inulin, chicory is the most common source. The roots of chicory look like small oblong-shaped sugar beets and their inulin content is more than 70% on dry substance, which is fairly constant from year to year (Franck, 2002).

The industrial production process of inulin involves the extraction of the naturally occurring inulin from chicory roots by diffusion in hot water, followed by purification and then spray-drying. High performance (HP) inulin is produced by removal of the fraction that have low DPs (degree of polymerization) after purification process (Franck, 2002; Shoaib et al., 2016).

Inulin has been a part of our daily food intake for centuries contributing to nutritional properties and exhibits technological benefits (Shoaib et al., 2016). Inulin is a prebiotic dietary fiber showing excellent properties as a carbohydrate-based fat substitute in relation to its ability to increase viscosity, form gels, provide mouthfeel and texture, and to increase water-holding capacity and thus presenting a good application potential in various food product formulations. Additionally, the incorporation of inulin in foods is known to reduce the risk of many diseases in human beings thus promoting health effects (Bodner and Sieg, 2009; Barclay et al., 2010; Rodriguez Furlán et al., 2014).

Chemical structure and physico-chemical properties of inulin

Inulin polymer consists of a long chain made up of 2-60 fructose molecules, which are connected by β-(2-1) bonds. The terminate fructose molecule is linked with a glucose molecule by α-(1-2) bond (Roberfroid, 1999, 2002; Bodner and Sieg, 2009). The degree of polymerization (DP) and branches have an effect on the functionality of inulin. Generally, while plant inulins are found to have chains incorporating 2-100 or more fructose units, chain length and polydispersity depending on plant species, microbial inulin has much larger degree of polymerization ranging from 10.000 to 100.000; furthermore, a bacterial inulin is 15% more branched than the plant inulin (Barclay et al., 2010; Shoaib et al., 2016). When inulin is extracted from the chicory root, it comprises a family of identical linear structures that differ in their degree of polymerization, ranging from 3 to 60 (Bosscher et al., 2006). The chemical structure of an inulin polymer is presented in Figure 1.
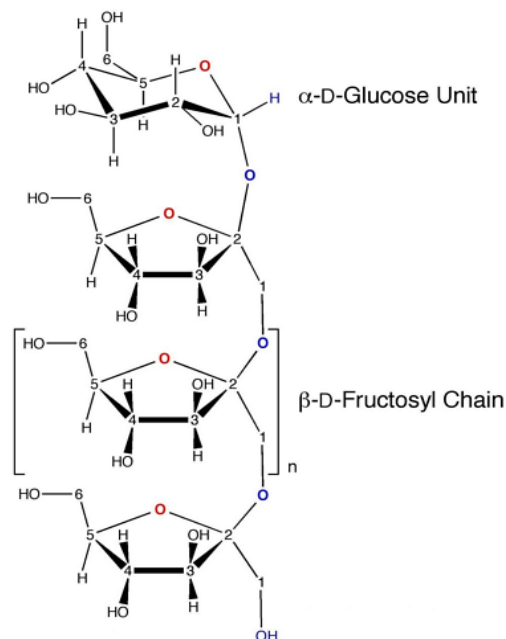
Figure 1. Inulin polymer (α-D-glucopyranosyl-[β-D-fructofuranosyl] (n-1)- D-fructofranoside) (Barclay et al., 2010).

Chicory inulin is a white, odourless powder with a high purity and well-known chemical composition. The physico-chemical properties of standard inulin and HP-inulin are presented in Table 1. Inulin has a bland neutral taste, without any off-flavour or aftertaste. Although standard inulin has a slight sweetness (10% compared to sugar), HP inulin has not due to removal of the fraction with a degree of polymerization lower than 10. Inulin combines easily with other ingredients and moderately soluble in water (Franck, 2002; Shoaib et al., 2016). Glibowski and Bukowska (2011) reported that in a neutral and alkaline environment, inulin is chemically stable independently of pH, heating time and temperature. However, chemical stability of inulin decreases in an acidic environment at pH ≤ 4 due to the heating time and temperature increase, thus limiting inulin applications in acidic foods, especially heated at temperatures above 60°C.

Table 1. Physico-chemical characteristics of chicory inulin (Franck, 2002).

	Standard inulin	**High performance (HP) inulin**
Chemical structure	GF_n ($2 \leq n \leq 60$)	GF_n ($10 \leq n \leq 60$)
Average degree of polymerization	12	25
Dry matter (%)	95	95
Inulin/oligofructose content (% on DM)	92	99.5
Sugars content (% on DM)	8	0.5
pH (10 % w/w)	5-7	5-7
Sulphated ash (% on DM)	< 0.2	< 0.2
Heavy metals (ppm on DM)	< 0.2	< 0.2
Appearance	White powder	White powder
Taste	Neutral	Neutral
Sweetness (*v.* sucrose=100%)	10 %	None
Solubility in water at 25°C (g/l)	120	25
Viscosity in water (5%) at 10°C (mPa.s)	1.6	2.4
Functionality in foods	Fat replacer	Fat replacer
Synergism	Synergy with gelling agents	Synergy with gelling agents

The utilization of inulin as a bulking agent, in particular as a fat replacer, is aided by its ability of water solubility. Parts of the molecular structure, specifically the hydroxyl groups, are more able to interact with water than other parts. This provides inulin with some surfactant character and it is able to form stable gels with water at concentrations of 13-50% (Barclay et al., 2010). When inulin is thoroughly dissolved in water or another aqueous liquid, with a shearing instrument like a rotor-stator mixer or high-shear homogenizer, it forms a particle gel network resulting in a white creamy structure (Franck, 2002; Shoaib et al., 2016). This unique property leads inulin gels provide considerable advantages, due to their similar textural characteristics to fat, allowing it to be used to replace fat, resulting in low fat foods that are palatable and have good mouth feel (Barclay et al., 2010). Franck (2002) emphasized that as far as fat replacement is concerned, HP inulin shows about twice the functionality of standard chicory inulin. Furthermore, inulin was reported as an ingredient working in synergy with most gelling agents such as gelatin, alginate, k- and i-carrageenans, gellan gum and maltodextrins (Franck, 2002).

Inulin gel is composed of a three-dimensional network of insoluble submicron crystalline in water (García et al., 2006). The most critical factors for gel formation of inulin are degree of hydrolysis, concentration and heating temperature (Kim et al., 2001; García et al., 2006). Kim et al. (2001) stated that gel formation could be a key step to produce carbohydrate based fat substitutes including inulin. In their study, they suggested that the heating-cooling process of inulin formed gels with stronger strength, smoother texture, more uniform and smaller particle size as compared to that obtained with a shearing process. In a study by Ronkart et al. (2010), it was reported that gelling properties of inulin-water systems were developed and the viscosity was increased when submitted to a microfluidization treatment, while the applied high shear stress did not induce a chemical composition change of inulin.

Health implications of inulin

Prebiotics are short chain carbohydrates which are capable of achieving the following criteria: (1) resistance to gastric acidity and mammalian enzymes, (2) susceptibility to fermentation by gut bacteria, and (3) ability to enhance the viability and/or activity of beneficial microorganisms (Bosscher et al., 2006; Al-Sheraji et al., 2013). Galactooligosaccharides (GOS), fructooligosaccharides (FOS) and inulin are the prebiotics most commonly known. While GOS are non-digestible and derived from lactose, inulin and inulin-type fructans are known as soluble dietary fibers (Al-Sheraji et al., 2013). The β-configuration of inulin makes it non-digestible to hydrolysis by human digestive enzymes, even those of the small intestine. Thus, undigested inulin reaches the large intestine, the most heavily colonized region of the gastrointestinal tract. Inulin is fermented by bifidobacteria and a wide variety of compounds that affect the

intestine and the systemic physiology is produced (Kim et al., 2001; García et al., 2006; Shoaib et al., 2016).

Dietary inulin is known to inhibit development of colon cancers in animal models. Similar tumor-inhibitory effects are seen with fermentation products of inulin, particularly the short chain fatty acids butyric and propionic acids, both of which inhibit growth of cancer cells (Roberfroid, 2002; Barclay et al., 2010).

Dietary inulin has been addressed to exert immune-modulatory effects and induces differentiation in several intestinal cell types to its effects on the gut flora (García et al., 2006; Barclay et al., 2010). Lowering the pH value of intestine, inulin provides assistance in relieving constipation and increasing stool load or rate, which is known as bulking effect (Shoaib et al., 2016). These modulatory effects of inulin possibly include indirect effects like changes in the composition of the intestinal flora, and the promoted synthesis of short chain fatty acids with immune-regulatory actions (Barclay et al., 2010).

Inulin has also been mentioned to reduce risk of cardiovascular diseases presumably by reducing serum concentrations of the proatherogenic molecule, p-cresyl sulphate, or by its favourable effect on plasma cholesterol and glucose levels (Barclay et al., 2010). One of the other impacts of inulin is the potential to decrease the risk of high triacylglycerol concentrations and blood lipogenesis, thereby reducing the risk of atherosclerosis. However, the mechanism that how inulin actually affects lipid metabolism in humans is still under discussion (Shoaib et al., 2016). An additional impact of dietary inulin is increasing calcium and magnesium absorption and bone mineralization in young adolescents (Roberfroid, 2002; Barclay et al., 2010; Al-Sheraji et al., 2013).

Besides the mentioned positive effects of inulin, the question is: are there any toxicity issues regarding this ingredient? Al-Sheraji et al. (2013) stated that numerous animal and human investigation studies had been performed to assess the possible intolerance caused by inulin and oligofructose, and the only biological effects observed had been attributed to their action as non-digestible, fermentable carbohydrates causing self-limited gastrointestinal distress (Barclay et al., 2010). Bodner and Sieg (2009) suggested utilization of lower doses of inulin in meat products to avoid digestive tolerance problems (consumption of inulin at levels higher than 4 g per serving can lead to the formation of unpleasant amounts of gas). Thus, depending on the fact that chicory fructooligosaccharides do not increase morbidity or mortality or cause reproductive or target-organ toxicity, these compounds are not mutagenic, carcinogenic, or teratogenic (Carabin and Flamm, 1999; Barclay et al., 2010).

Application opportunities of inulin in meat systems

Meat is a major source of high biological valued proteins and valuable nutrients. Besides essential amino acids and nutritive factors of high quality and availability; meat can be seen as an important source of many health-promoting compounds like peptides, bioactive hydrolysates, connective tissue components, nucleotides, phytanic acid, conjugated linoleic acids and antioxidants (Olmedilla-Alonso et al., 2013; Young et al., 2013; Hygreeva et al., 2014; Angiolillo et al., 2015). However, meat and meat products are also associated with nutrients and nutritional profiles often considered unfavorable including high levels of fat and saturated fatty cholesterol, sodium and caloric contents (Decker and Park, 2010; Hygreeva et al., 2014), which can increase the incidence of coronary heart disease, obesity, high blood cholesterol and certain types of cancer (Felisberto et al., 2015). Therefore, there has been a growing tendency to investigate the development of healthier meat product formulations. Some of the most investigated issues in relation to meat consumption and health aspects are means of reducing formation of unhealthy compounds like heterocyclic aromatic amines, reducing fat and cholesterol content and/or modification of lipid composition, reducing sodium nitrite and phosphate content, and incorporation of healthy ingredients like prebiotics, probiotics, synbiotics, vitamins and antioxidants (Olmedilla-Alonso et al., 2013; Young et al., 2013).

Fat is one of the essential components of meat products which contributes to the texture and flavour and increases the mouthfeel and juiciness, meanwhile it is responsible for cooking yield and characteristic aroma (García et al., 2006; Choi et al., 2013). Therefore, fat reduction implies technological and commercial problems in the manufacture of meat products with modified texture and sensory characteristics (García et al., 2006). It is of great importance that the ingredients used for fat replacement could compensate for the altera-

tions in quality parameters of low-fat meat products. Utilization of non-meat binders obtained from protein and carbohydrate sources is a common strategy for fat replacement in meat product formulations, which could mimic the behaviors of fat by increasing water binding, emulsification, gelling and thus improving product yield, texture and sensory quality (Brewer, 2012).

Inulin is currently used in several food systems as it can enhance the rheological and textural properties, improving the water-holding capacity and emulsion stability as a fat substitute and energy-reducing agent (Álvarez and Barbut, 2013). Inulin is considered to be a functional food ingredient and its utilization in food products include fat replacement and substitution (meat products, milk products, sauces, candies, etc.), reduction of caloric value (sugar-free chocolate, meat substitutes), water-holding ability (bakery goods), emulsification (margarine) and generally it is used to modify the texture and viscosity of foods (Franck, 2002; García et al., 2006; Glibowski and Bukowska, 2011; Shoaib et al., 2016).

The utilization of inulin can be considered a viable way to replace or reduce animal fat in meat products, by means of using natural ingredients as fat replacers (Bodner and Sieg, 2009; Álvarez and Barbut, 2013). Inulin is mentioned as a promising ingredient that could minimize the sensory and texture modifications caused by fat reduction, while contributing to the physiological benefits as a dietary fiber (García et al., 2006; Bodner and Sieg, 2009). Since inulin has the ability to form a stable gel network, it presents the advantage of being used to mimic some textural properties of fat and contributes a smooth, creamier and juicier mouthfeel when applied to low-fat meat product formulations (Frank, 2002; Bodner and Sieg, 2009). At the same time, inulin contributes few calories to the products, approximately 1 to 1.5 kcal/g (Coussement and Franck, 2001). Angiolillo et al. (2015) also stated that inulin have a neutral taste and is stable over a wide range of pH and temperature; thus presenting a great potential to be used for food applications. Bodner and Sieg

(2009) reported that in technological applications of inulin in meat systems, two usage strategies are possible: Pre-activation in water or addition at the beginning of the bowl chopper process. In case of utilization of crystalline inulin, 24 h are required for complete gelling.

Recent research on inulin incorporation in various meat product formulations is summarized in Table 2. The results of the studies so far have indicated that inulin has a great potential, improving overall quality of meat products. As could be seen in Table 2, in various emulsified, minced and fermented meat products, inulin was reported to provide advantages on reduction of animal fat meanwhile enhancing textural, sensory and technological quality parameters. In emulsified meat products, inulin could successfully enhance emulsion stabilization and cooking yield (Álverez and Barbut, 2013; Keenan et al., 2014) and protected texture and sensory parameters (Huang et al., 2011). In dry-fermented products, inulin was effective to cover physical, chemical, microbiological or sensory attributes during storage (Menegas et al., 2013).

In spite of all these advantages, some technological issues have been mentioned regarding the utilization of inulin. It was noted that inulin could result in a white exudate in vacuum-packaged frankfurters during storage, meaning that it was not fully capable of immobilizing water for the duration of shelf life. According to the researchers, based on its molecular weight and particle size, inulin responds to the osmotic pressure and migrated from the meat batter into the purge. To avoid this scenario, it was suggested to use lower doses of inulin in combination with other high water-holding fibers, such as wheat or citrus fiber (Bodner and Sieg, 2009). Angiolillo et al. (2015) found that in meat burgers using FOS and inulin with the oat bran decreased the cooking loss and shrinkage, due to the increased water binding properties of oat fiber combined with FOS and inulin. Felisberto et al. (2015) also suggested simultaneous addition of prebiotic fibers and cassava starch in meat emulsions, due to avoid low stability in the treatments containing inulin.

Table 2. Recent studies on utilization of inulin and supplementary ingredients in various meat products

Ingredient(s)	Research material	Research highlights	Reference
Wheat fiber, oat fiber and inulin	Chinese-style sausage	✓ The type and amount of dietary fiber used did not change chemical composition, colour and total plate counts. ✓ Addition of wheat and oat fibers hardened the texture, while added inulin did not influence the texture of the sausages. ✓ The sausage groups with added inulin had positive scores in sensory characteristics, showing no significant difference from the control group.	Huang et al., 2011
Inulin, β-glucan and their mixtures	Cooked meat batter	✓ Powdered inulin enhanced cook yield and provided advantages in emulsion stabilization, while emulsions containing gel inulin resulted in creamy and softer characteristics. ✓ Appropriate addition of inulin and β-glucan showed synergistic effects compensating for some of the changes brought about by fat reduction, and maintained several of the textural characteristics.	Álverez and Barbut, 2013
Inulin and corn oil	Dry-fermented chicken sausage	✓ The addition of inulin did not change the physicochemical and microbiological parameters. ✓ Inulin resulted in an altered texture profile and a tendency toward lighter and reddish coloration. ✓ Sausages with corn oil and inulin remained stable without a loss of physical, chemical, microbiological or sensory attributes during storage.	Menegas et al., 2013
Inulin	Breakfast sausage	✓ Increasing inulin inclusions decreased cook loss and improved emulsion stability, but also resulted in greater textural and eating quality. ✓ Hardness values increased with increasing inulin concentration, with panellists also scoring products containing inulin as less tender. ✓ Acceptable sausage formulations with low fat content were produced, which would contain sufficient inulin to deliver a prebiotic health effect.	Keenan et al., 2014
Inulin and bovine plasma proteins	Minced meat	✓ A fat reduction of 20-35% was supplied with products enriched with proteins and inulin. ✓ No change was observed in color, flavor or taste among the samples. ✓ In sensory test, the combination of plasma protein and inulin had the best acceptability with respect to consistency. ✓ Plasma protein and inulin usage decreased fat drain from the emulsion.	Rodriguez-Furlán et al., 2014

Fruktooligo saccharide (FOS), inulin and oat bran	Meat burger	✓ Combinations of both FOS and inulin, respectively combined with oat bran minimized the loss of prebiotic compounds during cooking of the meat burger samples. ✓ Addition of prebiotic in presence of foam enriched with oat bran improved the technological and sensory characteristics, giving products that appear to be very prized.	Angiolillo et al., 2015
Inulin, FOS, polydextrose, and resistant starch	Meat emulsion	✓ Low emulsion stability was observed, mainly in the treatments containing inulin and polydextrose. ✓ A compact and dense network was observed in microstructure in formulations containing inulin, due to its chain length, which could also affect the organoleptic properties. ✓ The simultaneous addition of a partial level of cassava starch and the prebiotic fibers was suggested to improve stability.	Felisberto et al., 2015

Conclusion

Today there has been a rising attention paid to specific types of beneficial ingredients like dietary fibers as the consumers are becoming more and more health conscious about foods. Inulin is one of these fibers offering positive effects in terms of product quality and health issues. Although the role of inulin as a nutritional and health beneficial ingredient has been explored in various researches, we specifically focused on its usage as a functional ingredient in meat product formulations within this review. Inulin presents excellent advantages in different meat products especially incorporated with other non-meat binders, and the impacts on quality attributes are mainly related with its physico-chemical properties. In connection with these data, further research is needed regarding meat product quality associated with inulin characterization and interactions with other compounds. In addition, since today there has been a rising demand on natural food ingredients, it is important to perform further research on the direct utilization of alternative natural sources of inulin, such as Jerusalem artichoke in meat product formulations.

Acknowlegments

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REVIEW ARTICLE   **DERLEME MAKALESİ**

ANISAKIASIS: PARASITIC HAZARD IN RAW OR UNCOOKED SEAFOOD PRODUCTS AND PREVENTION WAYS

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Abstract:

Parasitic infections related to the consumption of raw or uncooked seafood products have always been a concern for the consumers and for seafood economy. Anisakiasis is a serious zoonotic disease related with a wide range of syndromes in humans caused by member of Anisakidae. In last decade, an increasing number of anisakiasis disease have been reported, and this has been connected to the increase of globalized eating habits, ready to eat practices, the trend to avoid excessive cooked foods for nutrient preservation, consumption of fresh seafood for health reasons. Raw or slightly cooked ready-to-eat seafood products such as marinated, salted and cold smoked fish products, sushi and sashimi are the tool for transmission of *Anisakis* spp. larvae to human gastrointestinal system. As well as the factors that have yielded to an increase of the Anisakiasis cases, public health issues, anisakiasis symptoms, and methods to kill the *Anisakis* spp. larvae such as freezing, cooking, salting, marinating, irradiation, high hydrostatic pressure and chemicals have been reviewed in this study.

Keywords: Food safety, Parasitic hazard, Anisakiasis incidence, *Anisakis* spp.

Introduction

Parasitic infections regarding the eating of raw and uncooked seafood products have always been a concern for the consumers and for economy. In the last decade, there has been an increased number of reports regarding infections and/or allergic reactions in consumers owing to the increased awareness of doctors and an increased prevalence of these parasites in fish (Pozio, 2013). The main origin zoonoses related with the consumption of raw and uncooked seafood products are mainly due to the trematodes, cestodes and nematodes. Among the last mentioned, the anisakis species are the most common parasites from a sanitary way of thinking, since they are capable of inducing pathologies in consumers (Chai, Murrell, & Lymbery, 2005). Anisakiasis is a disease caused by nematodes having larval stages in aquatic hosts. The one of main nematode known to has caused disease in humans is *Anisakis simplex* (Beldsoe & Oria, 2001). Nematode of the genera *Anisakis* is parasite of sea mammals at the adult stage and of fish and cephalopods at the larval life stage (Anastasio et al., 2016; Pozio, 2013). Parasitic nematode, *Anisakis simplex,* reachs sexual maturity in the intestinal tract of marine mammals. The life cycle of anisakis species is shown at Figure 1. The life cycle of *Anisakis* spp. starts in the feces of an infected marine mammal (1). Marine mammals excrete unembryonated eggs (2). Eggs become embryonated in water and L2 larvae stage form in the eggs (3). After the L2 larvae hatch from eggs, they become free swimming. Free-swimming larvae are ingested by crustaceans and they mature into L3 larvae form (4). Infected crustaceans are eaten by fish such as rockfish, herring, mackerel, salmon and anchovy or squid (5). After the the host's death, larvae move to the muscle tissue, and through predation, the larvae are transferred from fish to fish this ways (6). Marine mammals such as dolphins, seals or humans may become infected from consuming the infected intermediate host (7). In humans, these worms do not mature, but the worms can migrate from the gastrointestinal tract, becoming embedded in the gastrointestinal mucosa and yielding tissue reaction and discomfort that is, gastric pain, diarrhea, vomiting (Beldsoe & Oria, 2001).
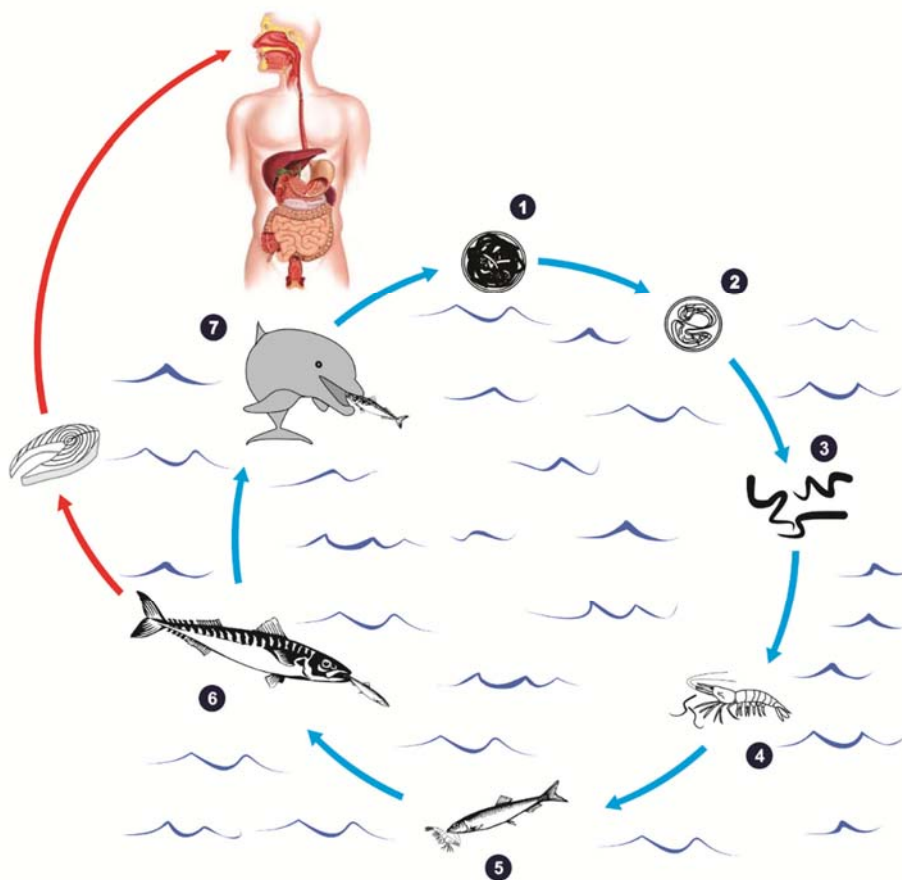
Figure 1. Life cycle of *Anisakis* spp. parasites.

The identification of *Anisakis* species is very difficult owing to the limited species-specific differences in morphological characters. Moreover, these differences are only visible in the matured worm and not in the larvae (Mattiucci et al., 2007). The larvae must have a size that makes them detectable and must be clearly differentiable from the tissues of the fish, even in the absence of optical instruments. In the literature, only two species were determined responsible for zoonotic forms: *Anisakis simplex*, known as "herring worm", and *Pseudoterranova decipiens*, known as "cod worm". However, the molecular studies based on genetic markers have reported that many morphospecies of *Anisakis* and *Pseudoterranova* include a certain number of sibling species with identical morphology, but different genetic make-up and geographical location. Currently, nine species of the *Anisakis* genus and six of the *Pseudoterranova* genus have been detected (D'amico et al., 2014).

Public health problems

Humans could become host if they eat raw, marinated or uncooked seafood that is infected at least one L3 viable which may then cause to a severe pathology, named as 'Anisakiasis' (Bao, Garci, Antonio, & Pascual, 2013). In general, anisakis larvae may be responsible for four forms of symptoms in consumers: gastric (i), intestinal (ii), ectopic (iii) and allergic (iv) symptoms. Additionally *Anisakis simplex* is now related with occupational seafood allergy (Audicana & Kennedy, 2008).

It known that anisakis nematodes could not be host at the larval life stages. It means that wide range of fish species can play a role as intermediate or host. Larval anisakis can infect through aquatic species by means of predation and may be transferred to larger predator. So, different aquatic species may play an important role in the spread of anisakis in the aquatic environment. Different aquatic species could be main source of infestation in humans, mammals and piscivorous birds (Shamsi, 2014). There is controversy about the effect and pathogenicity of anisakis worms on aquatic mammals and birds. While some researchers believe that infections with anisakis nematodes are not serious in aquatic mammal hosts (Geraci & Aubin, 1987), others have remarked that anisakis can be harmful in the alimentary tract of aquatic mammals (Abollol, Lopezz, Gestall, Benaventez, & Pascual, 1998; Jefferies, Hanson, & Harris,

1990). Anisakis have also been determined in terrestrial mammals, such as dogs and pigs, which are fed fish contains anisakis larvae with pathological changes resembling those found in aquatic mammal final hosts but differing in some aspects, such as in fewer macroscopic granulomata in pigs (Shamsi, 2014).

Over the last 30 years, there has been an increase in the reported prevalence of anisakiasis throughout the world. This increase may be due to a higher infection of captured fishes, improvements in the diagnosis of disease and the incorporation of foreign eating habits (Japanese sushi and sashimi) to food culture, and other typical seafood origin uncooked seafood snacks food dishes (marinated anchovies, etc.) (Bao et al., 2013). Several cases of infection have been reported in countries in which the consumption of uncooked fish is common (e.g. sushi in Japan, cod liver in Scandinavia, marinated fish Mediterranean countries), with a variety of clinical manifestations. Epidemiologically, *A. simplex* infections have been reported globally, with a marked prevalence in Japan. Indeed, Japanese cases alone account for more than 90% of all anisakiasis case reports (Hochberg, Hamer, Hughes, & Wilson, 2010), and some other cases are reported in Europe, in USA, and in Australia (Anastasio et al., 2016; Bucci et al., 2013; Cipriani et al., 2016; D'amico et al., 2014).

A recent survey of patients with generic gastrointestinal disorders in the United States reported that these symptoms were ascribable to parasitic diseases of aquatic origin, with such a frequency requiring preventive controls throughout the national territory (Hochberg et al., 2010). In Europe, the estimated incidence is almost 0.038% and most of the diseases have been reported in Spain, Italy, France, Netherlands and Germany (D'amico et al., 2014). Studies indicated that *A. simplex* was found in 39.4% of the fresh mackerel and 55.6% of blue whiting fish examined from different fish markets in Spain. In Italy, a few cases have been reported, particularly in related with the consuming of marinated anchovies (Bucci et al., 2013). The exact incidence is difficult to establish, but it seems to average 20 cases per country per year. In France, a report in 2003 estimated an incidence of 6 cases every year (D'amico et al., 2014).

The anisakiasis disease in developing countries such as Turkey has also not been considered to be a matter of great importance. Although there are some cases regarding occurrence of *Anisakis* spp. in fish, there is no report of human anisakiasis case

in Turkey. Studies showed that anisakis larvae commonly parasitized a great variety of fish from in Aegean and Mediterranean Sea coast of Turkey, excluding Black Sea, similarly to data reported in a number of surveys performed in most of the Mediterranean Sea along the European and African coasts (Keser et al., 2007; Meloni et al., 2011; Pekmezci et al., 2014; Serracca et al., 2013).

The factors that have led to an increase of the Anisakiasis cases over the past 30 years are many and interdependent. The food scares crises, for example the "mad cow disease" and the "avian influenza", which have shifted the orientation of consumers' attention towards proteins of aquatic origin, have increased the consumption of fishery products. Another factor to be taken into consideration is that the spread of ethnic food on the Western tables has led to the availability of a variety of Oriental dishes, especially Japanese (sushi), characterized by preparations of raw seafood. In fact, many Japanese restaurants are not really authentic, but managed by workers of different ethnicity, especially Chinese. These latter tend to increasingly convert their restaurant activities into Sushi Restaurants, offering cheaper products, often at the low quality. However, the lack of an exact knowledge on the microbiological and parasitic risks regarding dishes based on raw fish could lead to an inappropriate manipulation and treatment of the raw materials. (D'amico et al., 2014).

Symptoms of Anisakiasis

Human anisakiasis can be several forms. Clinically, following its penetration in the human gastrointestinal tract, A. simplex can cause gastrointestinal (classified as acute, chronic, or ectopic reactions) or allergy symptoms. The clinical symptoms vary depending on the organ infected and which Anisakis spp is ingested (Bucci et al., 2013).

The acute symptom typically involves the stomach and is characterized by abdominal pain, vomiting, and nausea within hours of the ingestion of Anisakis spp. contaminated food, mimicking an acute abdominal syndrome. In this type, an upper endoscopy performed within 12 h of the ingestion of larvae is essential to allow the localization and removal of A. simplex with a complete resolution (Sugimachi, Inokuchi, Ooiwa, Fujino, & Ishii, 1985). The chronic symptom is due to the localization of A. simplex in the intestinal wall. Typically, symptoms continue several months, with mild cramping abdominal pain, losing weight, and diarrhea, and it may be difficult to diagnose. A

subtype of this form is determined by the migration of the larvae beyond the gastrointestinal wall, with the localization of the worm in the peritoneal cavity or in solid or hollow organs, causing symptoms related to the involved organ (Bucci et al., 2013).

The allergic symptoms occur within several hours of after the consuming of contaminated fish. In gastro-allergic anisakiasis reactions may take place as secondary immune response after a previous infestation by live larvae. There is an ongoing discussion about whether primary sensitization by antigens from dead larvae can also happen. Four clinical allergic symptoms (gastric, intestinal, ectopic, and systemic) have been associated with Anisakis spp., and reactions may rely on the route of sensitization (Fæste et al., 2014). Several cases of anaphylactic shock, hypersensitivity reactions, urticaria, and angioedema have been represented in word association with the consuming of or re-exposure to contaminated fish (Bucci et al., 2013).

Most cases of anisakiasis around the world are because of the Anisakis or Pseudoterranova larval types (Shamsi, 2014). In Japan, it takes places most commonly as a gastric infection, while intestinal disease is more prevalent in Europe. In the United States, a recent report indicated that these symptoms were ascribable to parasitic diseases of fish origin, with such a frequency requiring preventive controls throughout the national territory (Hochberg et al., 2010). The Australian case of anisakiasis is due to Contracaecum larval type. Symptoms such as vomiting, diarrhea, sore throat, abdominal pain, nasal congestion, rhinorrhoea and cough l continue about 3 weeks until a larva is moved in a bowel motion Human infestations take place after consuming a infested seafood such as mackerel (Shamsi, 2014).

The endoscopic removal of the living larvae from the gastrointestinal wall is known as medical treatment of acute type of Anisakis worms. Conversely, the treatment of chronic and ectopic anisakiasis depends on the medical complications produced by the larvae, ranging from the need for surgical removal of the granuloma to the use of steroids to reduce local inflammation. Unfortunately, there is no effective pharmacological treatment to kill the larvae after eaten. The only protection against Anisakis spp. is the frozen storage and properly processing of seafood (Bucci et al., 2013). Most cases of anisakiasis have been related to the consumption of raw or uncooked seafood made with anchovy (Anastasio et al., 2016;

D'amico et al., 2014), salmon (Bao et al., 2013), herring (Cipriani et al., 2016), mackerel (Pekmezci, 2014), sardine (Rello, Adroher, & Valero, 2008), bonito/skipjack (Soewarlan, Suprayitno, & Nursyam, 2014), hake (Mattiucci, Abaunza, Ramadori, & Nascetti, 2004), mullet (D'amico et al., 2014), whiting (Llarena-Reino, González, Vello, Outeiriño, & Pascual, 2012), sea bass (Bernardi et al., 2011). *Anisakis* spp. including *Anisakis simplex* and *Pseudoterranova decipiens* are widespread in the raw or uncooked seafood products including marinated (Karl, Roepstorff, Huss, & Bloemsma, 1994), salted (Van Mameren & Houwing, 1970) and smoked (Beldsoe & Oria, 2001) fish products.

Inactivation methods of the parasites

Freezing

The current European Union ruling on food hygiene (the so called ''Hygiene Package'') takes into consideration the risk of the presence of parasites in fish products, and permits the consumption of fresh products only when they have been made safe through freezing (-20 ºC at the center of the product) or with other methods of proven efficacy, such as hot smoking at over 60 ºC or acidic marinating treatments sufficient to kill any parasites present (Reg. 853/2004, Section VIII, Chap. III, point D) (Brutti et al., 2010). Unlike bacteria, molds, and viruses, most parasites are easy to destroy by holding the raw material or finished product at freezing temperatures for a specified period of time; of course, this is dependent upon the core temperature of the food stuff (Beldsoe & Oria, 2001). The time required to reach the intended core temperature and fat content of the fish may affect the treatment. With regard to the type of parasite involved, some cestodes are more sensitive to freezing treatment than trematodes. According to the U.S. FDA, in order to inactive the nematode, the product may be subjected to various types of preventive treatments, which provide different time/temperature combinations, including:

i- Freezing at -20 °C followed by a storage minimum 7 days at -20 °C (or lower);

ii- Freezing at -35 °C (or lower) followed by a storage at -35 °C (or lower) for 15 h;

iii- Freezing at -35 °C (or lower) followed by a storage at -20 ° C (or lower) for 24 h.

Freezing, as a preventive treatment, is a procedure expressly required by law and, according to the provisions about the correct information to consumer; the data regarding the process have to accompany the product up to the retail sale. However, this information is usually neglected, especially in catering and food service. Defrosted fish yield a mistrust among consumers, showing a reluctance to purchase and consume it. In this regard, "defrosted" term should not be served on fishery and aquaculture products subjected to a preventive treatment for food safety and health purposes (D'amico et al., 2014).

Salting and Marinating

Although *A. simplex* are sensitive to salt, the high salt concentrations and times needed for its elimination make salting an inadequate method of inactivation (Beldsoe & Oria, 2001). Some European countries, such as Spain and France, have determined the technical conditions of salting and acidic marinating to kill the larvae of the parasite, thus excluding the preventive freezing of the products. In Spain, the Scientific Committee of AESAN, asserted that freezing is not necessary for those fishery products that reach a concentration of NaCl above 9% for at least six weeks, between 10 and 20% for four-five weeks or more than 20% for at least three weeks. With regard to salting, the French Food Safety Agency (AFSSA), reported that in traditional preparations and for small quantities salinity levels of 20% result in the inactivation of the parasite within 21 days, while concentrations of 15% require 28 days. AFSSA also indicated that, according to some scientists, fish marinated with 10% acetic acid and 12% salt, maintained for 5 days at 4 °C, are not hazardous to health as well as marinated seafood products within 12% salt and 6% of acetic acid for 13 days at 4 °C (D'amico et al., 2014).

In the last decade some novel techniques were developed in order to inactivate anisakidae larvae in seafood products, such as irradiation and high hydrostatic pressure even if these methods have showed some negative effect on sensorial properties of these products (Giarratana, Muscolino, Beninati, Giuffrida, & Panebianco, 2014).

Irradiation

The freezing treatment can also be shifted by either irradiation or treatments with high pressures. Irradiation of seafood is an effective method of inactivating nematodes. Earlier studies reported that in order to inactive *Anisakis* spp. in salted herring, doses of as high as 6 to 10 kGy were necessary

(Mameren & Houwing, 1968). Similarly, *A. simplex* larvae was found to be highly resistant to irradiation doses of 2 kGy or 10 kGy in another study (Beldsoe & Oria, 2001). Unfortunately, the irradiation treatment procedure used to kill the nematodes seems to induce negative changes in the organoleptic characteristic (Farkas, 1998). In addition, in the EU the use of ionizing radiation for seafood products is not approved by most of the Member States (D'amico et al., 2014).

High Hydrostatic Pressure

High hydrostatic pressure has been used for treating food to extend its shelf life and has been indicated to inactive *Anisakis* spp. larvae, although the common usage of high hydrostatic pressure technology in food have been to inhibit endogenous enzymes and inactivate microorganisms (Vidacek, de las Heras, Solas, Rodriguez Mahillo, & Tejada, 2009). The hydrostatic pressure required to inactive *Anisakis* spp. larvae is generally much lower than that used for inactivating the microorganisms. Pressures of 200 MPa for 10 min and 207 MPa for 3 min were reported to kill 100% isolated *Anisakis* spp. larvae and larvae in fish muscle (Molina-García & Sanz, 2002). Regardless of its effect on the larvae, hydrostatic pressure may yield some negative sensorial and functional changes in the fish muscle, perceived as changes in texture, color and lipid oxidation, which will differ according to the pressure/time conditions applied (Vidacek et al., 2009).

Chemical or Natural Additives

Concerning chemical additives, only the hydrogen peroxide was recognized for its effect against *Anisakis* spp. larvae, although its use it is not allowed in the European Community. Recently, several studies had reported a significant effect against the L3 larvae of *Anisakis* spp. exerted by various natural products including essential oils of different terrestrial plants such as thyme (*Thymus vulgaris*), chamomile (*Matricaria chamomilla*), tea tree (*Melaleuca alternifolia*), peppermint (*Mentha piperita*), (Barros et al., 2009; del Carmen Romero, Valero, Martín-Sánchez, & Navarro-Moll, 2012; Giarratana et al., 2014; Hierro et al., 2004).

Conclusion

Surveillance of anisakiasis and monitoring of *Anisakis* spp. over decades has demonstrated that seafood related parasite has become a major contributor to human fish-borne disease because of its hospitalization rates related to the organism's invasive qualities. Food attribution studies combined with data from food research, risk assessments and scientific expert opinion may help to determine where the greatest risks are for anisakiasis. The scientific data produced are useful as specific inputs for shelf life at manufacturing and information for consumers around food choice, especially for susceptible populations. However, due to the ready to eat and snack food types and consumption patterns differ around the world, *Anisakis* spp. inspection and control researches may need to be performed on regional basis. Most of the countries have established parasites regulations, industry guidance documents, and consumer educational practicing strategies but impact are still so low. At present, anisakiasis diseases seem to be a lower priority compared to other public health problems but its surveillance in many parts of the world is very limited. The seafood-borne parasitic diseases are of great importance for public health, and the above-mentioned precautions should be taken before serving of raw or uncooked seafood to consumption. It suggests that the critical control points at the Hazard Analysis Critical Control Point (HACCP) programmer should be properly reviewed to reduce the risk of anisakis induced allergies for seafood consumers.

Acknowlegments

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ORIGINAL ARTICLE/**ORİJİNAL ÇALIŞMA**

FULL PAPER   **TAM MAKALE**

THE EFFECT OF ACTIVE AND VACUUM PACKAGING ON THE QUALITY OF TURKISH TRADITIONAL SALTED DRIED FISH "ÇİROZ"

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Abstract:

Changes in biochemical and sensory attributes of Turkish traditional salted dried fish products "çiroz" during storage packaged by oxygen absorber and vacuum were investigated. For this purpose, total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), TBA index values (TBA-i), free fatty acids value (FFA) and peroxide value (PV), sensory attributes and microbiological analyses were carried monthly during storage. According to sensory analysis and TVB-N results, the samples of çiroz packaged active and vacuum, spoilt on the 6th months at cold storage. TBA-i, FFA and PV of fatty oxidation parameter showed similarity in both groups of samples. Microbiological findings did not exceed limit values during storage in both groups. When comparing two types of packaging, it is concluded that the active package, which is a new approach, may be an alternative to vacuum packaging. This was determined by the study; oxygen-absorbing systems could be used to pack fish products sensitive to oil oxidation.

Keywords: Salted dried fish, Active packaging, Oxygen absorber, Vacuum packaging, Quality

Introduction

Salting and drying are one of the oldest methods used in fish preservation. These methods are effective in the production of traditional fish products. These methods can be used individually or in combination. The preservative effect of salting and drying is mainly due to the decrease in water activity. Thus, the growth of many spoilage organisms is prevented (Schormüller, 1968; Sikorski et al., 1990; Tülsner, 1996; Albarracín et al., 2011). Salted cod is a traditional fish product of choice in the northern Atlantic region, whereas salted sardine salted anchovy are traditional fish products preferred in the Mediterranean and the Black Sea. Salting and drying techniques are used together in some fish products. The product called klippfish is one of these. Klippfish are popular most particularly in Germany, Norway, Newfoundland, Iceland, and the Faroe Islands. This product can be made with whitefish; fatty fish is not preferred. The fish is beheaded and eviscerated, dry salted and the fish was sun- dried on rocks or wooden frames. Today drying process is usually done indoors by electrical heating. Especially preferred for klippfish is cod, may be produced from other whitefish, such as Pollock, haddock, blue whiting, ling and tusk (Schormüller, 1968; Xie and Myrland, 2010; Ceballos, 2012). Traditional salted dried fish products made in the Aegean and Marmara regions of Turkey and Greece are called çiroz. Unlike klippfish, fatty fish is preferred. Fatty fish such as Atlantic horse mackerel (*Trachurus trachurus*), Mediterranean horse mackerel (*Trachurus mediterraneus*)*,* chub mackerel (*Scomber japonicus*) and Atlantic mackerel (*Scomber scombrus*) are caught after egg casting (when they have lower fat content), are eviscerated, dry salted, then dried in slightly windy, low humidity air (Özden, et al., 2001; Kolcuoğlu, 2013). The products are often vacuum or air packed and stored at chilled temperatures.

Active packaging is a new concept of food packaging, was developed in response to changes in current consumption and market trends and is designed to improve fresh and processed sea food product quality and safety (Quintavalla & Vicini, 2002; Kerry et al., 2006). Active packaging refers to the incorporation of certain additives into packaging systems with the aim of maintaining or extending product quality and shelf-life. Packaging may be termed active when it performs some desired role in food preservation other than providing an inert barrier to external conditions (Floras, 1997; Ahvenainen, 2003). Active packaging systems involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation, and finally antimicrobial systems (Coma, 2008). Oxygen absorbing systems provide an alternative to vacuum and gas flushing technologies as a means of improving product quality and shelf life (Kerry et al., 2006)

To the best of our knowledge, there is no information in the literature on the use of active packaging for the preservation of dried fish products. The aim of this study was to compare the effects of vacuum and active packaging on the shelf life and quality of salted dried fish stored at cold storage.

Materials and Methods

Çiroz was prepared from Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758). Atlantic mackerel (246.9 ±60.1 g and 28.04 ±2.28 cm) was obtained from Bozo Balıkçılık, İstanbul fish market. The product preparation process is shown in Figure 1.

The products (75 g per pouch) was placed in low density polyethylene/ethylene vinyl alcohol/low density polyethylene pouch (LDPE/EVOH /LDPE; thickness: 55 µm, O_2 transmission: 5 $cm^3/m^2/day/24$ h; vapour permeability: 7.50 $g/m^2/24$ h). Samples was divided into two groups. In first group (AP) was added inside the package three OxyFree 504 type iron based O_2 absorber (Süd-Chemie Company, İstanbul, Turkey). The first group pouches were heat sealed using a Henkovac model vacuum sealer (ML's-Hertogenbosch Nederland). The second group (VP) were vacuum packaged using a Henkovac model vacuum machine and all samples were stored at 2 ±2°C.
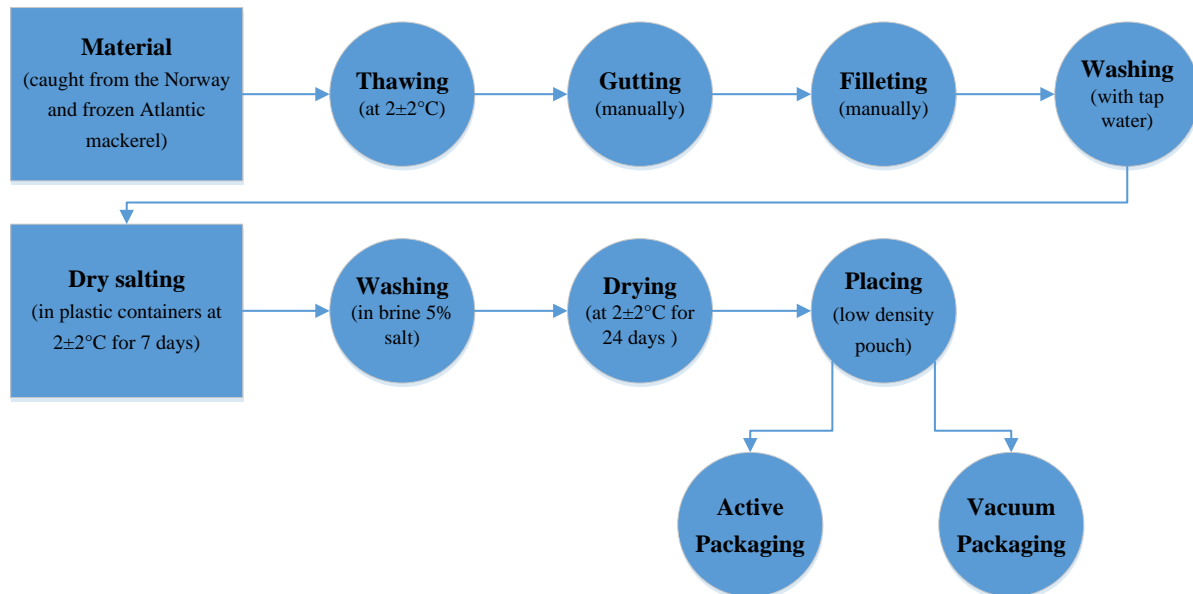
Figure 1. The production process of Turkish traditional salted dried fish "çiroz"

Sensory analysis: Sensory testing was performed after the fallowing desalting procedure. The fillets were soaked in drink water (ratio 1:5) for 30 min. This process is repeated three times. The fillets were drained and served with 2% citric acid, lemon and olive oil. The attributes of çiroz were evaluated by a panel of five experienced judges on each month of sampling in individual booths under controlled conditions of light, temperature and humidity. Sensory analysis was performed using the methods of Erkan and Bilen (2010). Desalted çiroz samples were assessed on the basis of appearance, odour, taste and texture characteristics using a nine-point descriptive scale. A score of 9-7 indicated ''very good'' quality, a score of 6.9–5.0 "good or acceptable quality", a score of 4.9–1.0 "unacceptable quality". The appearance, odour, taste and colour of the samples were evaluated, and the mean values of these attributes were presented.

Chemical analysis: Moisture, protein, ash, water and salt content of product were measured by Mattissek et al. (1992) method. Total volatile basic nitrogen (TVB-N, mg/100g fish flesh), trimethylamine nitrogen (TMA-N, mg/100g fish flesh), TBA index values (TBA-i, mg/ malondialdehyde (MDA)/kg), free fatty acids value (FFA, oleic acid %) and peroxide value (PV, meq O_2/kg fish flesh) was determined according to the method described by Erkan & Bilen (2010).

Microbiological analysis

Samples (25g) obtained from çiroz fillet, were transferred aseptically to a Stomacher bag (Seward Medical, London, UK) containing 225 mL of 0.1% peptone water (Merck, 107228) and homogenized for 60 s using a Lab Blender 400, Stomacher at high speed (Stomacher, IUL Instrument, Spain). For microbial count, 0.1 mL samples of serial dilutions (1:10, diluents, 0.1% peptone water (Merck, 107228, Darmstadt, Germany) of fish homogenates were spread on the surface of agar plates. Plate count agar (PCA) was used for psychrotrophic bacteria and incubated at 7°C for 10 days. Anaerobic counts were determined by PCA incubated under anaerobic conditions (with 5 % CO_2 incubator, HF 90 model, Shanghai, China) at 30°C for 24-48 h. Extremely halophilic bacteria were enumerated in halophilic agar (25 % NaCl) incubation after at 30ºC for 7 day. Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample. Thus, the detection limit of psychrotrophic, anaerobic and extremely halophilic bacteria counts was <1.00 log cfu/g. All the analyses were performed in duplicate. For anaerobic sulphite-reducing *Clostridium* count, 25 g of sample were homogenized and incubated at 30°C for 14 days in Differential Reinforced Clostridial *Broth* (*DRCM*, Merck 1.11699) under anaerobic condition. Results were expressed as log MPN/g of samples (Bell et al., 2005).

*Statistical analysis***:** For each group, data from two independent replicate trials were pooled and the mean values and standard deviations were determined. Differences between groups were determined by Tukey test and were considered to be significant when p<0.05.

Results and Discussion

Acceptability scores for sensory properties of active and vacuum packaged çiroz samples decreased (significant, p<0.05) during the storage. The acceptability limit for sensory scores was reached after 5 months for the two groups' samples. Not significant difference (p>0.05) was observed between the groups during the storage. There was no reported scientific study for the sensory acceptability of çiroz or dried fish samples. It is reported that the shelf life dried fish products is about one year, although it varies according to the salting and drying process, fish species, packing type and storage condition (Tülsner, 1994). The shelf life of active packed fish products were found higher than that of aerobically packaged products (Mexis et al., 2009).

The amount of salt used in dry salting was twenty percent of the total fish weight. The salt content in product and the salt content in tissue water of product also moisture content of salted fish was measured after seven days of salting process, were found as 12.25 ±0.5 %, 24.13 ±0.3 % and 35.5 ±0.5 %. If the amount of salt in the tissue water of product is above 24 percent, it is defined as heavily salted product (Tülsner, 1996). The salt content in product, the salt content in tissue water of product and moisture content of products were found as 15.0 ±0.7 %, 41.09 ±0.5 % and 18.5 ±0.2 % after 24 day of drying process. For fresh fish and çiroz samples, the chemical composition values were determined as follows: moisture 50.1 ±1.1 mg/100g and 18.5 ±0.2 mg/100g, ash 1.3 ±0.1 mg/100g and 5.2 ±0.3 mg/100g, total protein 20.4 ±2.0 mg/100g and 38.28 ±2.0 mg/100g, total fat 25.08 ±0.8 mg/100g and 37.42 ±0.6 mg/100g. This is in agreement with the conclusions made by literature data (Guizani et al., 2008; Selmi et al., 2010; Bae et al., 2011).

The relationship between results of TVB-N and sensory data was found excellent for vacuum and active packaging çiroz samples. Sikorski et al. (1990) reported that the limit of acceptability for fatty fish was 20 mg TVB-N/100 g of flesh. The release of total volatile bases increased up to 21.20 ±3.40 mg/100 g for çiroz in vacuum packaging and 17.08 ±3.48 mg/100 g in active packaging at the last day of sensory acceptability for each packaging condition. The statistical analysis of TVB-N data showed that not significant differences (p>0.05) were found between packaged in active and vacuum çiroz samples after 5 months of storage. Similarly, TMA-N value of samples increased throughout storage. However, there were no significant differences (P>0.05) between the TMA-N values at every stages of storage of çiroz in vacuum packaging and active packaging. The limit values of TMA-N were reported as 5 mg/100g for fatty fish species (Sikorski et al., 1990). This limit value was not exceeded throughout the storage in active and vacuum packaged samples.

Atlantic mackerel, which is a raw material of çiroz, has polyunsaturated fatty acids and are sensitive to peroxidation. Free radicals react with oxygen to produce fatty acid peroxides. The fatty acid peroxides are free radicals which can attack another lipid molecule, resulting in peroxide and a new free radical. The primary product of lipid oxidation is the fatty acid hydroperoxide, measured with peroxide value (PV) (Hamre et al., 2003). As seen in table 1, initial PV values were 5.69 ±2.14 meq O_2/kg for çiroz packaged in oxygen absorber and 5.83 ±2.30 meq O_2/kg for çiroz packaged in vacuum. The maximum values of PV were found 39.66 ±1.04 meq O_2/kg for çiroz packaged in oxygen absorber in the sixth month of storage and 41.94 ±1.33 O_2/kg for çiroz packaged in vacuum in the five month of storage. Similar results were reported by Selmi et al. (2010) for dried fish products.

As a consequence of oxidative spoilage, lipid hydroperoxides are formed, which, in turn, are unstable and decompose to aldehydes, ketones, alcohols, acids or hydrocarbons. These so-called secondary oxidation products can change food quality parameter, namely, colour, texture, flavour and odour (Andersen et al., 2007; Azad Shah et al., 2009; Christensen et al., 2011). One of the most important products of seconder oxidation is malondialdehyde (MDA), MDA has often been used as marker of oxidative damage in fatty foods. The most widely used method for determination of MDA is the spectrophotometric determination of the pink fluorescent MDA-thiobarbituric acid (MDA-TBA) complex produced after reaction with 2-thiobarbituric acid (TBA) at low pH and high temperature (Hamre et al., 2003). The TBA

value is an important parameter in determining lipid oxidation (Insausti et al., 2001). At the beginning of the storage, TBA values were found as 9.39 ±0.63 and 9.47 ±0.73 mg malonaldehyde/kg fish flesh, for the first 5 months of storage in all samples showed a continuous increase, after 5 months the value of TBA showed declines were observed (Table 1). It has been reported that in many literatures the negative changes in taste and smell become apparent when the TBA value reaches its maximum value (Guilluén-Sans and Guzmán-Chozas, 1998). Similar results were found in this study. The significant decrease in sensory values (acceptable limit) and the maximum value of TBA were measured at the fifth month of storage.

Glycerides, glycolipids and phospholipids in fatty fish muscle are hydrolysed by lipases to free fatty acids, which then undergo further oxidation to produce low molecular weight compounds, such as aldehydes and ketones. These compounds are responsible for off-flavour and off-odour and taste of fish and fish products (Hamilton et al., 1997). Initial values ranged from 2.10 to 2.50 (% of oleic acid) while final values ranged from 4.08 to 3.35 for çiroz packed in oxygen absorber and vacuum, respectively. These results indicate that there is a relationship between FFA release and loss of freshness. In the present study, the production of peroxide and free fatty acid was also slower in çiroz samples packaged in oxygen absorber than çiroz samples packaged in vacuum.

Table 1. Changes in sensory and chemical properties of Turkish traditional salted dried fish "çiroz"

Storage time (Monthly)		0	1	2	3	4	5	6	7
Sensory score	AP	8.75 ±0.15[A]	7.43 ±0.12[A]	6.85 ±0.13[A]	6.58 ±0.15[A]	5.80 ±0.12[A]	**5.20 ±0.08**[A]	4.95 ±0.11[A]	4.35 ±0.13[A]
	VP	8.45 ±0.25[A]	7.30 ±0.08[A]	6.65 ±0.10[A]	6.38 ±0.19[A]	5.75 ±0.05[A]	**5.10 ±0.05**[A]	4.85 ±0.08[A]	4.13 ±0.10[A]
TVB-N (mg/100g fish flesh)	AP	2.64 ±0.51[A]	4.93 ±0.92[A]	8.71 ±2.02[A]	11.75 ±3.53[A]	13.60 ±2.64[A]	17.08 ± 3.48[A]	**21.30 ±3.50**[A]	22.43 ±3.48[A]
	VP	2.95 ±0.37[A]	10.01 ±0.09[B]	12.36 ±2.11[B]	14.90 ±0.29[B]	18.74 ±0.24[B]	**21.20 ±3.40**[A]	22.43 ±3.92[A]	23.92 ±2.06[A]
TMA-N (mg/100g fish flesh)	AP	1.64 ±0.16[A]	2.03 ±0.15[A]	2.44 ±0.01[A]	2.72 ±0.04[A]	3.09 ±0.47[A]	3.20 ±0.64[A]	3.39 ±0.76[A]	3.64 ±1.04[A]
	VP	1.73 ±0.07[A]	2.16 ±0.28[A]	2.61 ±0.10[B]	3.00 ±0.37[A]	3.19 ±0.52[A]	3.28 ±0.61[A]	3.61 ±0.81[A]	4.16 ±1.30[A]
PV (meq O$_2$/kg fish flesh)	AP	5.69 ±2.14[A]	15.25 ±2.94[A]	23.45 ±1.39[A]	26.66 ±2.89[A]	28.31 ±2.96[A]	32.59 ±2.92[A]	**39.66 ±1.04**[A]	12.30 ±2.50[A]
	VP	5.83 ±2.30[A]	17.37 ±3.97[A]	24.95 ±0.37[A]	31.17 ±0.56[B]	34.42 ±0.26[B]	**41.94 ±1.33**[B]	15.64 ±1.30[B]	8.37 ±2.17 [B]
TBA-i (mg malonaldehyde/kg fish flesh)	AP	9.39 ±0.63[A]	11.30 ±0.98[A]	11.82 ±0.66[A]	12.77 ±0.28[A]	13.20 ±0.23[A]	**15.84 ±0.34** [A]	8.96 ±1.19[A]	7.93 ±2.00[A]
	VP	9.47 ± 0.73[A]	11.67 ±0.85[A]	13.27 ±0.04[B]	13.58 ±0.17[B]	14.18 ±0.36[B]	**17.32 ±0.08**[B]	9.45 ±0.12[A]	6.85 ±0.80[A]
FFA (g oleic acid/100g fish flesh)	AP	2.10 ±0.64[A]	2.32 ±0.46[A]	2.58 ±0.28[A]	2.80 ±0.22[A]	3.34 ±0.08[A]	4.03 ±0.22[A]	**4.51 ±0.22**[A]	4.08 ±0.32[A]
	VP	2.50 ±0.10[A]	2.59 ±0.14[A]	2.70 ±0.26[A]	3.13 ±0.04[B]	3.75 ±0.06[B]	**4.27 ±0.15**[A]	3.93 ±0.03[B]	3.35 ±0.15[B]

In these hard salted products, halophilic bacterial growth is possible and extremely halophilic bacteria should be analysed on these products (Tülsner, 1994). In this study, the psychrotrophic, anaerobic and extremely halophilic bacteria count all during storage was not exceeded over 4 log cfu/g in both groups. Anaerobic sulphite-reducing *Clostridium* count was determined <1 log cfu/g in packaged vacuum and oxygen absorber çiroz samples during the storage. Hernández-Herrero et al., (1999) reported a similar microorganism load for extremely salted anchovy products.

Conclusion

As a result, it can be said that the active packaging systems using oxygen absorber in the packaging of çiroz products may be an alternative to vacuum packaging. Quality losses due to fatty oxidation are important for a traditional salted dried fish product "çiroz". While the preferred vacuum packaging system for fatty oxidation requires equipment, active packaging systems by oxygen absorbers, an alternative packaging approach, appear to be an alternative to packaging of fish products sensitive to fatty oxidation.

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REVIEW ARTICLE

DERLEME MAKALESİ

LAKTULOZ ELDESİ VE TESPİT EDİLMESİNDE KULLANILAN YÖNTEMLER

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Öz:

Laktuloz, laktozun izomerizasyonu sonucu oluşan bir disakkarit olup, çok değerli bir fonksiyonel maddedir. Teknolojik olarak öneminin yanı sıra laktulozun birçok fonksiyonel özelliği de bulunmaktadır. İnce bağırsak mukozasında herhangi bir değişikliğe uğramadan kalın bağırsağa geçerek öncelikle bifidobakterler gibi metabolizma için yararlı olan probiyotik bakteriler tarafından kullanılmakta ve bu bakterilerin gelişimini teşvik etmektedir. Ayrıca patojen bakterilere karşı probiyotik bakterilerin etkisinin laktuloz katkısı ile ciddi düzeyde desteklendiği pek çok araştırmada bildirilmektedir. Bu çalışmada laktulozun tespiti ve eldesinde kullanılan yöntemler ile çeşitli süt ürünlerinde laktuloz miktarlarına ilişkin bilgilere yer verilmiştir.

Anahtar Kelimeler: Laktuloz, Süt ürünleri, Laktuloz tespiti, Laktuloz eldesi

Abstract:

PRODUCTION OF LACTULOSE AND METHODS USED TO DETERMINATION

Lactulose is a disaccharide, which is occurred as a result of isomerization of lactose and a very valuable functional subtance. There are also several functional properties of lactulose as well as technological importance. It goes through the colon without any changes in the intestinal mucosa and used by probiotic bacteria such as bifidobacteria which are useful for metabolism. Also it encourages of these bacteria. In addition, it has been reported in many studies that lactulose has significant support effect on probiotics against pathogen bacteria. In this study, some informations are given about obtaining and determining methods of lactulose and also the amount of lactulose in various dairy products.

Keywords: Lactulose, Dairy products, Determination, Obtain of lactulose

Giriş

Laktuloz (4-O-b-D-galaktopiranosil-D-fruktofuranoz); bir disakkarit türevi olup, galaktozun fruktoza β (1-4) glikozidik bağ ile bağlanması sonucu oluşur. İlk kez 1930 yılında Montgomery ve Hudson tarafından elde edilmiştir, doğada doğal olarak bulunmamaktadır. (Özden, 2005; Montilla vd., 2005a; Boitz ve Mayer, 2015). Ampirik formülü, molekül ağırlığı ve erime noktası sırası ile $C_{12}H_{22}O_{11}$, 342.30 g/mol ve 169°C' dir (Nooshkam ve Madadlou, 2016a). Laktulozun enerji değeri düşüktür (2.0 kkal/g), suda çözünürlüğü iyidir, tekstür ve stabilizasyon üzerine etkisi bulunmaktadır.

Laktuloz, ince bağırsak mukozasında herhangi bir değişikliğe uğramadan kalın bağırsağa geçerek öncelikle bifidobakterler gibi metabolizma için yararlı olan probiyotik bakteriler tarafından kullanılmakta ve bu bakterilerin gelişimini teşvik etmektedir. Patojen bakterilere karşı probiyotik bakterilerin etkisinin laktuloz katkısı ile ciddi düzeyde desteklendiği bildirilmektedir. Bu nedenle fonksiyonel değeri yüksek bir prebiyotiktir (Akalın, 2002; Kavas ve Kavas, 2011).

Laktuloz, sakkarozun % 60-80'i oranında tatlılığa sahip olması nedeni ile gıda sanayinde kullanım alanı bulunmaktadır (Nooshkam ve Madadlou, 2016b). Fonksiyonel özellikleri dışında teknolojik olarakta önemlidir ve yoğun olarak kullanılan bir bileşendir. Yapılan bir çalışmada laktuloz ilaveli bebek mamaları ile beslenen bebeklerin bağırsak florasının anne sütü ile beslenen bebeklerin florasına yakın olduğu belirtilmektedir. Laktuloz, özellikle fonksiyonel özellik kazandırmak amacı ile bebeklerin beslenmesinde Japonya ve Avrupa'da tercih edilmektedir (Özden, 2005). Laktozdan biraz daha tatlı olması nedeni ile diyabetik ürünlerde, laktasif etkili şurupların yapımında ve tıpta "hepatik ensefalopati" olarak adlandırılan rahatsızlığın ve kabızlığın tedavisinde kullanılmaktadır (Nahla ve Musa, 2015). Bağırsaktan toksik maddelerin atımını kolaylaştırdığı gibi bağırsak içeriğini asitleştirerek amonyağın emilimini azaltmakta ve bağırsaktan atılmasını kolaylaştırmaktadır (Akın ve Erden 2002). Laktuloz hem gıda hem de ilaç sanayisinde kullanılan bir maddedir.

Laktuloz Miktarının Tespit Edilmesi

Laktulozun beslenme için önemli bir bileşen olması nedeni ile gıdalardaki miktarının tespit edilmesine yönelik analitik metodlar geliştirilmiştir.

Yapılan araştırmalarda laktulozun miktarının belirlenmesinde genellikle yüksek performanslı sıvı kromatografisi (Silveira vd., 2015), gaz kromatografisi (Padilla vd., 2015), ince tabaka kromatografisi (Flick vd., 1987) ve benzer teknolojiler kullanılmaktadır. Ayrıca seliwanoff reaktifi kullanılarak sonuç veren spektrofotometrik yöntemler de mevcuttur (Amine vd., 2000).

Süt, tüketici sağlığı ve raf ömrü açısından farklı süre ve sıcaklıklarda ısıl işleme tabi tutulmaktadır. Uygulanan ısıl işleme bağlı olarak süt bileşenlerinde bazı değişiklikler oluşmakta veya yeni bileşenler ortaya çıkmaktadır. Bu yeni bileşenlerden bir tanesi laktozun ısıl işlem neticesinde izomerizasyonu sonucu sütte oluşan laktulozdur. Oluşan laktuloz miktarı uygulanan ısı yoğunluğu ile doğru orantılıdır ve bu durum nedeni ile süt işleme teknolojisinde kalite kontrol aşamasında kullanılabilecek bir indikatördür (Silveira vd., 2015).

Pastörize, sterilize ve UHT sütlerde laktuloz miktarları yapılan farklı çalışmalarda belirlenmiştir. Pappas vd. (2015) laktulozun pastörize sütte bulunmadığını, direk ve indirek ısıtma tekniğine bağlı olarak UHT sütlerde sırası ile 50-850 ve 190-830 mg/L, sterilize sütlerde ise 1080-1400 mg/L oranında bulunduğu bildirmektedir. Marconi vd. (2004) ise klasik sterilize sütte 744.0 mg/L, indirekt yöntem UHT sütte 341.0 mg/L, direkt yöntem UHT sütte 165.0 mg/L, yüksek sıcaklık uygulanan pastörize sütte 58.0 mg/L, düşük sıcaklık uygulanan pastörize sütte 3.50 mg/L, kaynatılmış sütte 107.0 mg/L laktuloz saptamışlardır. Farklı bir çalışmada UHT sütlerde depolama süresince laktuloz miktarının arttığını belirlenmiştir (Elliott vd., 2005).

İçme sütü teknolojisinin yanı sıra sütten üretilen ürünlerde laktuloz miktarlarına ilişkin araştırmalar bulunmaktadır. Pastörize süt ve süte eşdeğer oranda sulandırılarak hazırlanan süt tozlarının laktuloz oranlarının saptandığı araştırmada pastörize sütte 7.0-28.3 mg/L, süt tozunda 16.1-26.5 mg/L düzeyinde laktuloz tespit edilmiştir (Rafael vd., 1996). Süt ve süt tozu örneklerinde ısıl işlemin düzeyine göre laktuloz miktarı değişim göstermektedir. Yüksek basınçlı sıvı kromatografisinde yapılan laktuloz tayininde 100°C sıcaklığa kadar yapılan ısıl işlemlerde laktuloz tespit edilmediği, 100°C'nin üzerindeki sıcaklıklarda laktuloz oluşumunun görüldüğü ve 130°C'den sonra maksimum seviyelere (130°C'de 551 mg/L, 140°C'de

1549 mg/L) çıktığı belirtilmektedir (Sakkas vd., 2014).

Yeni doğan bebeklerin beslenmesinde kullanılan mamaların bileşiminde süttozu ve peyniraltı suyu tozu vb. kullanılmaktadır. Gonzáles vd. (2003) süt ve peyniraltı suyu ile zenginleştirilmiş yeni doğan formülasyonlarında enzimatik olarak laktuloz miktarını belirlemişlerdir. Peyniraltı suyu ile zenginleştirilmiş formülasyonlarda laktuloz miktarının yüksek olduğu (97-312 mg/L) gözlemlenirken, zenginleştirme yapılmayan sütlerde ise düşük olduğu (29-108 mg/L) belirlenmiştir.

Yapılan bir çalışmada pastörize, uzun raf ömürlü ve UHT krema örneklerinde laktuloz miktarları sırası ile 29 ±10, 56 ±41 ve 201 ±24 mg /L olarak bulunmuş olup laktulozun bu ürünlerde ısı yükünü değerlendirmede güvenilir olarak kullanılabileceği belirtilmiştir (Boitz ve Mayer, 2015). Ayrıca ısıl işlem görmüş ve dondurularak kurutulmuş süt örneklerinde laktuloz miktarını herhangi bir kimyasal ön hazırlık gerektirmeyen dağınık yansıtıcılı kızılötesi Fourier dönüşümlü spektroskopi (DRIFTS) ile belirlemek için basit, zararsız, ucuz ve kısa sürede sonuç veren bir yöntem geliştirilmiştir (Pappas vd., 2015).

Görüldüğü üzere süt ve süt esaslı ürünlerde işleme yöntemlerine göre laktuloz oluşmakta ve miktarı uygulanan prosese göre değişmektedir. Son yıllarda laktuloz oluşumu ve miktarının farklı metotlarla tespit edilmesine yönelik yapılan araştırmaların arttığı da gözlenmektedir.

Farklı Yöntemlerle Laktuloz Eldesi

Laktulozun oluşumu için farklı yöntemler bulunmaktadır. Laktuloz, temel olarak kimyasal izomerizasyon (asit veya baz kullanarak) ya da enzimatik sentez yöntemleri ile elde edilebilmektedir (Sitanggang vd., 2015). Endüstriyel olarak laktuloz üretimi Lobry de Bruyn-Alberda van Ekenstein (LA) olarak belirtilen özel bir reaksiyonla gerçekleştirilmektedir. Bu reaksiyon, uygun pH, basınç ve sıcaklıkta alkali ortamda ilerlemektedir. Katalizör olarak sodyum, potasyum ve kalsiyum hidroksitler, tersiyer aminler, magnezyum oksit, sodyum ve potasyum karbonatlar kullanılmaktadır (Nooshkam ve Madadlou, 2016a). Bu katalizörlerin yanı sıra amonyum karbonat kullanımının alternatif çevre dostu bir kimyasal olacağı bildirilmektedir (Seo vd., 2016). Alternatif katalizör madde bulmak üzere yapılan araştırmaların son yıllarda artış gösterdiği görülmektedir. Kullanılacak olan katalizörün düşük maliyetli, ortamdan kolay uzaklaştırılabilir, çevre dostu, güvenli ve

toksik olmaması gerekmektedir (Panesar ve Kumari, 2011).

Nooshkam ve Madadlou (2016a) bileşiminde yaklaşık % 96 kalsiyum karbonat, % 1 magnezyum karbonat, % 1 kalsiyum fosfat, organik madde ve su içermesi nedeni ile yumurta kabuğunun, laktozdan laktulozun izomerizasyonunu sağlamak amacı ile kalsiyum karbonat bazlı katalizör olarak kullanılabileceğini belirtmişlerdir. Yumurta kabuğunun katalizör olarak kullanıldığı araştırmada laktozun laktuloza dönüşüm verimi % 17.3 olarak bulunmuştur. Alkali koşullarda borat ve alüminatlar gibi kompleks reaktiflerin kullanılması ile laktuloz verimi % 70-80'lere çıkabilmektedir. Fakat bu reaksiyonlarda fazla miktarda katalizör ihtiyacı olmakta ve karışımdan katalizörlerin ayrılması nispeten zor olmaktadır. Bu nedenle yumurta endüstrisinde önemli bir atık olan yumurta kabuğunun değerlendirilmesine ve çevre dostu bir katalizör kullanımına olanak sağlayan bu tür çalışmalara rastlanmaktadır (Montilla vd., 2005b; Corzo Martinez vd., 2013). Aynı araştırmacılar yapmış oldukları farklı bir çalışmalarında mikrodalga destekli laktuloz izomerizasyon yönteminin verimi artırdığını bildirmişlerdir (Nooshkam ve Madadlou, 2016b).

İyon değiştirici reçineler kullanılarak laktozun laktuloza izomerizasyonu da sağlanabilmektedir. Bu işlemde OH^- iyonlarının reaksiyon çözeltisindeki ve reçinedeki değişimi kullanılmakta ve aynı prosesle son üründeki demerilizasyon işlemi de yapılabilmektedir. Bu yöntemin avantajı, izomerizasyon işlemi için katalizör madde ilavesine ve son ürünün saflaştırılmasında boya maddesi kullanımına gerek olmamasıdır (Panesar ve Kumari, 2011).

Yapılan çalışmalardan görüldüğü üzere laktulozun saflaştırılmasında temel olarak çöktürme, iyon değiştirici reçineler, kristalizasyon, santrifügasyon; reaksiyon ortamının rengini gidermek için ise aktif karbon işlemlerinden yararlanılmaktadır. Bu yöntemler tek başına veya kombine şekilde kullanılabilmektedir. Yöntemde kullanılan materyale göre işlem basamakları değişiklik göstermektedir. Bu işlemlerde temel amaç en uygun şartlarda en yüksek saflığı yakalamaktır (Panesar ve Kumari, 2011).

Laktulozun eldesinde endüstride yoğun olarak kullanılan yöntem kimyasal izomerizasyondur. Fakat bu yöntemin bazı olumsuz yönleri bulunmaktadır. Bunlardan bazıları renkli yan ürünlerin

açığa çıkması, atık yönetimi ile ilgili sorunların olması ve spesifik reaksiyonun zayıf olmasıdır. Kimyasal izomerizasyon yöntemi temelde; nötralizasyon, katalitik seperasyon ve deiyonizasyon aşamalarını içermektedir (Sitanggang vd., 2015). Kimyasal izomerizasyon yönteminin olumsuz yönleri olması nedeni ile alternatif olarak kullanılan enzimatik sentez yönteminin daha çevre dostu olduğu ve ürün saflaştırmasının daha zahmetsiz aşamalardan oluştuğu belirtilmektedir (Sitanggang vd., 2015, Wang vd., 2015).

Enzimatik sentez yönteminde genellikle β-glikozidaz ve β-galaktozidaz enzimleri kullanılarak laktuloz üretimi gerçekleştirilmektedir. Laktulozun enzimatik olarak sentezlenmesi laktozun transgalaktosilasyonu yolu ile gerçekleşmekte, bu reaksiyonda enzimler biyokatalizör, fruktoz galaktosil akseptörü olarak kullanılmaktadır (Nath vd., 2016). Laktuloz sentezinde kullanılan enzimler mikroorganizmalardan, bitki ve hayvanlardan elde edilebilmektedir. Bununla birlikte mikroorganizmalardan elde edilen enzimlerin diğerlerine göre daha yüksek verim sağladığı bildirilmiştir (Nath vd., 2016). Enzimatik yolla laktuloz sentezi; serbest enzim, immobilize enzim ve bütün mikroorganizma hücresinin kullanımı ile gerçekleştirilebilmektedir. Verimin düşük olması ve yüksek maliyet, enzimatik sentez yönteminin olumsuz yönleri olarak görülmektedir (Wang vd., 2015).

Yapılan çalışmalardan görüldüğü üzere her iki yöntemin de avantaj ve dezavantajları bulunmaktadır. Her iki yöntem ile ilgili özellikle son yıllarda yapılan çalışmalar artarak devam etmektedir. Farklı kimyasallar, enzimler veya kombine yöntemler kullanılarak yüksek saflıkta laktuloz eldesi için ekonomik ve çevre dostu yöntemler geliştirilmektedir.

Laktulozun enzimatik yolla sentezlenmesinde kullanılan enzim başta olmak üzere, enzimin çalışmasını etkileyen pH, sıcaklık, basınç vb. gibi proses şartları, laktuloz oluşum miktarını ve verimini etkilemektedir. Bu nedenle bu konularda farklı araştırmacılar tarafından çok sayıda çalışmanın yapıldığı görülmektedir. Song vd. (2013a) tarafından laktuloz sentezi için geliştirilen sabit yataklı reaktörde kesikli ve sürekli olarak üretim yapılmış, bu kapsamda peyniraltı suyu hammadde olarak kullanılmış, reaktöre immobilize edilen β-galaktosidaz enziminin tekrar kullanılabilirliği araştırılmış, üretim için gerekli optimum fruktoz konsantrasyonu %5'lik olarak tespit edilmiş ve laktuloz sentezinin kinetik parametreleri belirlenmiştir. Guerrero vd. (2015) *Aspergillus oryzae* suşundan elde edilen β-

galaktosidaz enzimini hem serbest olarak kesikli sistemde hem de immobilize ettikten sonra tekrarlamalı kesikli sistemde laktuloz eldesi için kullanmışlardır. Sonuç olarak tekrarlamalı kesikli sistemin laktuloz üretiminde belirgin artış sağladığı ve biyokatalizör birim kütlesi başına toplam verimliliğin arttığını belirtmişlerdir.

Koyun peynirinden izole edilen *Kluyveromyces lactis* ve *Kluyveromyces marxianus* suşlarından elde edilen β-galaktozidaz enzimleri ile peyniraltı suyundaki laktozun transgalaktosilasyon ile prebiyotik karbonhidratlara (tagatoz, laktuloz, galakto-oligosakkarit) dönüşümünün sağlandığı araştırmada kullanılan yöntemin prebiyotik oligosakaritlerin üretiminde kullanılabileceği bildirilmiştir (Padilla vd., 2015).

İmmobilize edilmiş β-galaktosidaz ve glukozizomeraz enzimi ile laktuloz eldesinde peyniraltı suyu tozu, sodyum fosfat tamponlu çözeltisi kullanılmış; laktoz derişimi, sıcaklık, tampon çözeltinin iyonik kuvveti ve immobilize enzimlerin kullanım oranları gibi reaksiyon koşulları optimize edilmiş olup ayrıca enzimlerin tekrar kullanılabilirliği araştırılarak enzim sarfiyatı asgari düzeye indirilmiştir. Araştırma sonucunda sürekli üretimde akış hızının arttıkça laktuloz sentezinin azaldığı gözlemlenmiş olup, 1.0 µL/dk hızda maksimum laktuloz sentezi (1.42 g/L) belirlenmiştir (Song vd., 2013b). Wang vd. (2015) laktuloz üretimi için biyokatalitik bir metot geliştirmişlerdir. Bu amaçla etanol ile muamele görmüş *E.coli* hücreleri içerisinde rekombinant sellobiyoz 2-epimeraz enzimini kullanmışlardır. Biyokatalizör olarak bütün hücre kullanımının enzim saflaştırma aşamalarında avantaj sağladığı bildirilmiştir. Bu avantajlardan bazıları, endüstriyel olarak biyolojik dönüşüm prosesinin daha basit, kolay işlenebilir ve ekonomik olması olarak belirtilmiştir. Bu işlem ile %65.1 laktuloz dönüşüm verimi ile maksimum ürün verimi 390.59 g/L olarak bulunmuştur.

Laktoz elektro katalitik izomerizasyon yöntemi ile de laktuloza dönüştürülebilmektedir. Sıcaklık, elektrik akım yoğunluğu ve reaktör tasarımı laktuloz sentezinin verimini etkileyen önemli faktörlerdir. Aissa ve Aider (2014) tarafından yapılan çalışmada % 10'luk laktoz çözeltisiyle en yüksek laktuloz verimi (%30.19) ve son ürün saflığı 10°C'de, 200 mA elektrik şiddetinde elde edilmiştir. Yapılan farklı bir çalışmada laktozun laktuloza elektro-izomerizasyon ile dönüşümü farklı laktoz derişimlerinde ve elektrik akımlarında peyniraltı suyu kullanılarak denenmiştir. Araştırma sonucunda laktoz konsantrasyonunun izomerizasyon

verimine önemli ölçüde etki etmediği, ancak laktoz çözeltilerindeki verimin aynı oranda laktoz içeren peyniraltı suyundan daha fazla olduğu gözlemlenmiş olup bunun sebebinin ise peyniraltı suyunda bulunan minerallerden kaynaklandığı düşünülmüştür. Bu yöntemde en etkili parametrenin ise elektrik alan yoğunluğu (200 mA) olduğu tespit edilmiştir (Aider ve Vidal, 2012).

Geleneksel termal yöntemlere alternatif olarak kullanılan ultrasonik ses yöntemiyle laktuloz eldesinde çevre dostu ve kaliteli ürün elde edilebilmesinin yanında bu yöntemin ısısal yöntemlerle kıyaslandığında yüksek pH'lı tampon çözeltiler içinde iyi sonuç verdiği tespit edilmiştir. Diğer taraftan bu yöntemin ısısal muameleyle kombinasyonunda verimin arttığı belirlenmiştir (Martinez vd., 2014).

Laktuloz sentezlenmesinde verim ne kadar önemli ise elde edilen ürünün saflaştırılması, saflaştırma aşamaları ve bu aşamadaki maliyette önemlidir. Laktuloz üretimi sırasında glukoz, galaktoz ve epilaktoz gibi bazı yan ürünler kayda değer miktarda ortaya çıkmaktadır. Düşük miktarda bile oluşan galaktoz, tagatoz, epilaktoz ve formik asitin laktulozu indirgeyebildiği ifade edilmekte fakat bu durumun pH ve sıcaklığı düşürerek giderilebileceği belirtilmektedir (Nath vd., 2016).

Sonuç

Laktuloz, insan beslenmesinde, probiyotik gıdalarda özellikle bebek mamalarında ve ilaç sektöründe yoğun olarak kullanılan fonksiyonel bir prebiyotiktir. Süt endüstrisi ilerlemiş ülkelerde süt ve yan ürünlerinin değerlendirilmesi son derece hassas bir biçimde yapılmakta, her bileşeni değerlendirilmektedir. Isıl işlem görmüş süt ve süt ürünlerinde, peyniraltı suyu gibi yüksek miktarda laktoz içeren sütçülük yan ürünlerinde teknolojik proses esnasında kendiliğinden laktuloz oluştuğu bilinmektedir. Ayrıca laktuloz temel olarak kimyasal izomerizasyon (asit veya baz kullanarak) yada enzimatik sentez yöntemleri ile elde edilebilmektedir. Hem fonksiyonel hem de teknolojik açıdan hem fonksiyonel hem de teknolojik açıdan öneme sahip olan laktulozun en ekonomik ve çevre dostu işlemlerle yüksek verimde elde edilmesi ve saflaştırılması için yapılan çalışmaların devam etmesi gerektiği düşünülmektedir.

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