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Review paper

Food production and security in the area of Serbia: historical background and current situation

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A b s t r a c t: Today's civilization finds itself facing existential problems in the survival of life on planet Earth. The aim of this paper is to point out the importance of food production from the moment when humans became hunter-gatherers until today, when there is talk about food security from the aspect of providing sufficient amounts of food for present and future generations. The paper presents the production of food in Serbia since the arrival of Slavic tribes on the Balkan Peninsula up to modern times. The paper also points to the historical development of food production and the necessity for socially responsible behaviour to preserve the resources we have for future generations.

Keywords: *Serbia, history, food production, food safety.*

Introduction

Today's civilization is facing existential problems of survival, but the most important of them is food. From the time when man moved from a hunter-gatherer lifestyle, and especially from the time of the first organized civilizations (Egypt, China), the creation of food supplies for lean times has been a concern (*Nićiforović-Babac*, 2009). Humans consciously and instinctively care about food supplies and food security. To describe the situation in which sufficient amounts of food are available, we use the term "food security", which was defined as: "When all people at all times have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (*FAO,* 2002; *Food Security*, 2006). According to data from the Food and Agriculture Organization of the United Nations (FAO), 850 million people in the world (11% of the world's population) are chronically malnourished, and in one year, 5,642 million children under the age of five, and 959,000 children between the ages of five and fourteen die of hunger (*Fan and Polman*, 2014; *Hug et al.*, 2017).

Increasing agricultural production and reducing the amount of food wasted (lost) are ways to reduce the number of malnourished populations and mortality due to starvation. According to the FAO definition, famine is a condition when a person does not eat enough food to meet their energy needs (1800 kcal per day) for a healthy and active life. Famine can also be defined as malnutrition in situation when a person does not intake enough macronutrients (proteins, fats, carbohydrates) or micronutrients (vitamins, minerals). Today, two billion people in the world are considered to be malnourished, which is often referred to as "hidden malnutrition". When it comes to macronutrients, the most common cause of malnutrition is insufficient protein intake. Micronutrient deficiencies usually involve vitamin A, iron, iodine and zinc. For society, famine and malnutrition have undoubted economic significance through health care expenditure, reduced working capacity etc. According to FAO estimates, famine and malnutrition in the world is expected to end between 2025 and 2030 (*Fan and Polman*, 2014).

People and life on earth

Since the beginning of human species, people's life on planet Earth has depended on the gifts of nature. More than two million years ago, archaic human species ate plants and their fruits, as well as animal meat. This, long before the beginning of agriculture, was the time when humans separated from other animals, i.e.,

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stood up (*Homo erectus*), began to use their hands to make tools, and moved from gesticular to mutual verbal communication. Later, in the period before around 200,000 years ago and according to skeletal remains, anatomically modern people mostly lived in small groups of around 20 members (*Baltić and Bošković*, 2015; *Hailicwkes*, 1966; *Vuković*, 2015).

This long historical period was when great progress was achieved in tool making, perfecting and gaining experience in hunting, and acquiring knowledge of the plant world and its seasonal changes. At this time, humans experienced the connection between diet and health (*Baltić et al*, 2010). The first farmers began to domesticate animals and cultivate plants 10,000 to 12,000 years ago. Nowadays, *Homo sapiens* cannot survive without agriculture. The world's first civilizations were associated with agriculture, i.e., cultivation of wheat and barley in the area south of the Caspian Sea, Kurdistan and Levant around 8,000 B.C., rice in China about 6,000 B.C. and corn in Central America, cultivated by Aztecs by 7,000 B.C. (*Nićiforović-Babac*, 2009; *Heun et al*, 1997; *Yval*, 2014). There is no doubt that agriculture changed the world in all aspects of its existence, both natural and social.

Food security on the Balkan Peninsula

The Balkan Peninsula was inhabited by Neanderthal people more than 25,000 years ago, in the Pleistocene. This is evidenced by the traces of people in numerous caves in the lands now encompassed by Serbia, of which the cave near the village of Gradac, under Jerina hill (Batočina) and Risovača on Venčac (Arandjelovac) are most often mentioned. These people were hunter-gatherers, and they mastered the skills to process the stones and bones that they used in hunting. When climate change reduced their food resources, they left their home ranges (more expressed in the ice age periods) (*Gavela*, 1962; *Srejović*, 1978; *Ilić*, 1995). The Holocene, the next geological epoch in the development of the countries in the Balkans, including Serbia, was marked by the cultures of Lepenski vir (9000-4500 BC), Starčevo (5300-4400 BC) and Vinča (4400-3200 BC), which belong to the Neolithic (*Srejović*, 1978; *Borić and Dimitrijević*, 2007; *Garašanin*, 1973; *Filipović et al.*, 2018; *Diklić*, 2017). The most distinct traces of these cultures are in the places that bear their names, but there are also some in other parts of the Balkan Peninsula, i.e., north and south of the Sava and Danube rivers. From the time of Theodosius II in the 5th century to the dynasty of Heraclius in the 7th century, Slavs migrated to the area of the Eastern Roman Empire — Byzantium. They came from their ancient homeland which was the area of the Carpathians, i.e., around the Dnieper and Bug rivers (*Ostrogorski*, 1996). The Eastern Roman Empire fell in 1453 after centuries of fighting with the Ottoman Empire, but also as a consequence of the conflict of peoples within the empire itself. That was time when the Serbian states, which were part of Byzantium, also disappeared (*Ostrogorski*, 1996).

Since their arrival on the Balkan Peninsula, and even during the medieval period, Serbs adhered to their traditional diet. Perhaps, the best indication of this is the fact that when they migrated to the Balkans, they took with them to the area a primitive breed of pig known as Šiška (*Hrasnica et al.*, 1964). There is not much information about how they lived or how they ate in the first centuries after they settled in the Balkans. However, it must be assumed that in the new conditions, they acquired new habits and had to adapt to a new environment. The area they came from was rich in water and fish. In such an environment, it is quite understandable that they were skilled boat builders and were good fishermen. For these strong people, the Balkan Peninsula, in relation to their homeland, was arid. However, it cannot be claimed that in their ancient homeland they lived only from fishing. Those vast lowland areas were suitable for livestock, agriculture and hunting (*Ivanović et al*., 2012). In Serbia at the time of early Slav settlement, the most important food resources were oak forests, because of acorns and their importance in pig nutrition, and mountain pastures. In the time of Emperor Dušan (14th century), deforestation was banned and restrictions on ploughing forest pastures began. Cattle, sheep and horses were fed on mountain pastures in summer, and in valleys and plains in winter. Pigs lived permanently in the oak and beech forests and were not moved.

In medieval Serbia, livestock breeding was the main branch of the economy. Practically the entire population was engaged in livestock breeding and/or agriculture. Emperors, nobles and the priesthood directed Serbs to engage in various forms agriculture, like fruit growing, beekeeping and their associated crafts, and the people named Vlachs to engage in livestock breeding. In medieval Serbia, the term Vlachs referred to livestock breeders of different ethnic origins (Serbian, Romanian, Greek). They lived in *katuns*, seasonal huts and settlements mentioned in documents in the 12th and 13th centuries. Thus, the population of Serbia was divided into those who were mainly engaged in plant-based agriculture and those whose main activity was animal husbandry. Villag-

es were located on the plains or at the foot of mountains, while the *katuns* were in the mountains to take advantage of summer pastures (*Cvijić*, 1991).

There are no data about the number of livestock in Serbia in the early 12th century. However, in the medieval Serbian state, livestock breeding was the most advanced form of agriculture from the time of King Milutin (1282–1321), and reached a peak during the rule of Emperor Dušan (1331–1355) (*Radojević et. al,* 2011). Livestock was the people's basic capital, and the animals served instead of money, as a means of payment, and even for the payment of fines. The importance given to livestock breeding and grazing is also shown by the fact that several articles in Dušan's Code regulate this area (*www.iksi.ac.rs*).

Sheep and pigs were the most commonly raised livestock, followed by cattle, horses, goats, buffaloes and poultry. The flocks of sheep were huge, as evidenced by the fact that in 1398, the Ottomans captured about 5,000 sheep from Dubrovnik merchants. In the area of Branković in 1455, the Ottomans registered over 25,000 pigs, i.e., more than two pigs per family (*Spremić*, 1994). However, most of the large animals were cattle. Emperors, nobles and monasteries kept the most cattle on their estates. Most often, one ordinary household had two oxen, two to four cows, three to four pigs and 10 to 20 sheep and goats. There were also poultry, especially chickens. In summer, small and large livestock animals were kept near rural households, and in winter, the animals were moved indoors, into barns (*Blagojević*, 1989). Domestic pigs reached weights of about 100 kilograms, and cows gave two to three litres of milk per day.

Households were engaged in agronomy, raising wheat, barley, rye and millet and in olericulture, raising vegetables such as cabbage, onions and beets. In Serbia at that time, the semi-nomadic Vlachs engaged in livestock breeding and production of food of animal origin. Between 20 and 100 families lived in each *katun* settlement at altitudes over 1,000 meters above sea level (*Cvijić,* 1991). The main product of these livestock breeders was cheese made from sheep's milk (*Tomić*, 1922). In addition to sheep, professional breeders kept a small number of cattle and horses; the horses served as pack animals. The Arbanassis people, mentioned in the 11th century, led a similar life to the Vlachs. The use of pastures and forests to feed livestock was taxed and paid for in grain, animals, meat, honey, wax or cheese. During Emperor Dušan's rule, one head of cattle was given to over-winter 100 cattle or horses on manorial lands, and four sheep with a lamb were paid for over-wintering 100 sheep (*Jiriček*, 1923). Pigs were fed on acorns in oak forests, and the ruler took from each pig owner a fee (tax) called *zhirovina* that amounted to 10% of the number of pigs. Charters from that time mention the *svinjski desetak* (literal translation; about ten for pigs). The fee paid to nobles or monasteries for the use of their forests and/or pastures could also be in money (*Jiriček*, 1923; *Arsić*, 2010). When livestock were sold, the ruler's animals (their own or those obtained as a *desetak*) had to be sold first. The same principle was used when they sold meat or meat products. The meat was sold fresh, and professional butchers are mentioned in most Serbian cities. There were also regulations around the sale of meat. Despot Stefan Lazarević, in Novo Brdo, also prescribed the price of meat (*Marković*, 1985). In addition to fresh meat, canned, salted and dried meat was also traded. Salted and dried pork was exported, and Dubrovnik merchants were noted traders of these products. By then, the majority of the population were used to bacon and lard in their diet. In medieval Serbia, besides meat from domestic animals, venison and other game meats were also used in the diet. Wild boar, chamois, deer, rabbit, wild duck, wild goose, partridge and pigeon were hunted (*Mišić*, 1992). Also, beekeeping existed as a professional occupation. Rulers, nobles and monasteries had their own beehives. Beekeeping was also taxed, so there was a *pčelinji desetak*. In 1455 in the Branković area, the Ottomans listed all the beehives; that list shows that many households had beehives, with some of them having more than a dozen beehives (*Novaković*, 1912).

Livestock products were mainly meat, especially salted pork, and cheese, then leather, wool and horns. In this period, new cities like Novo brdo, Prizren and Prishtina were just beginning to develop. The majority of the population lived in the countryside, so agriculture and livestock farming, along with mining, formed the basis of the economy. Serbia had significant quantities of grain and was a well-known grain exporter; again, the best buyers were Dubrovnik merchants. Grain was ground in mills owned by rulers, nobles, monasteries, cities, or also ordinary people (*Jiriček*, 1922).

Fish was an important item in the diet of the population of Serbia. In medieval Serbia, fish originated from rivers, lakes and sea. The Despot Stefan Lazarević Law on Mines was partly dedicated to the quality of fish, but it also referred to the trade of meat (*Radojčić*, 1960). The monasteries were the biggest consumers of fish, especially during religious holidays (when a form of fasting was required that denied meat from warm-blooded animals but allowed fish consumption). Fish was often on the menu of nobles and rulers. Monasteries and rulers had their own

fish ponds. While freshwater fish were mostly traded fresh, marine fish were usually salted to prevent quick spoilage. Seafoods like octopus, cuttlefish and shellfish were also valued (*Spremić*, 2004).

It cannot be said that in the medieval period there was always enough food for everyone in Serbia; famine years occurred in almost every decade. Famine years, during which people ate acorns, roots, grasses, leaves and tree bark, were mostly caused by droughts, hail, floods or frosts. After these types of natural disasters that were the cause of food insufficiency, war was the next most common reason for famine years. Usually, opposing sides fought during the summer, and then an army would destroy the grain or deplete the population by encircling and besieging the cities. In the early medieval period, both in Europe and in Serbia, corn, potato, sunflower, sugar beet and soybean were unknown crops. There is no doubt that these crops have significantly changed agricultural production and, thus, the diet of people in the "Old World". Corn was brought to Europe in the late 15th or early 16th century. Sunflowers have been grown in Europe since the 17th century, although more significant areas were sown after the Second World War. Soybeans have been grown extensively in America since the 19th century, and in Europe after the Second World War. Beans were brought from the Americas in 1542, and in the 17th century, were introduced from Italy to the Balkan Peninsula. Potatoes were brought to Europe from South America in the 16th century. Dositej Obradović introduced potatoes to Serbia during the First Serbian Uprising (1804–1813) (*Baltić and Marković*, 2017).

Food production in Serbia in the 19th century

At the beginning of the 19th century, there had been no significant changes in agriculture in Serbia since medieval times. Livestock breedingwas still the main activity of the population. However, the structure of agricultural production was changing over time, i.e., agronomy finally prevailed, and livestock movements gradually decreased and have practically disappeared today. Significant changes in agricultural production took place in Serbia at the end of the first half of the 19th century. From the medieval period until the 19th century, the largest part (75%) of Serbia south of the Sava and Danube rivers was forested. In the second half of the 19th century, there was a great reduction in the area under forests. People cut down forests and turned the lands into pasture and arable land for agriculture (*Lazarević and Lazarević*, 2016).

Agronomy then became the basic occupation in agriculture. As it was still being developed, though, it could not provide meaningful surpluses of corn, wheat, barley, oats or rye. However, the favourable configuration of the land, its soil structure and the climate enabled farmers to produce vegetables, fruit and livestock, which allowed them to provide for most of their personal food needs.

Agriculture brought Serbia increasing economic benefits over time. Serbia became independent as a state, so surplus agricultural production, especially livestock (pigs, cattle), was exported to Austria-Hungary. In 1875 during the rule of Milan Obrenović, Serbia had 1,352,500 inhabitants in five regions: Šumadija, Mačva, Rujno, Stari Vlah and Raška-Timočka Krajina-Braničevo, and in the city of Belgrade. Most (90%) of the population, was engaged in agriculture. The first livestock inventory in independent Serbia was made in 1867 during the reign of Mihailo Obrenović, when it was determined that there were 5,284,103 livestock animals. Of that number, sheep constituted over 50.7%, pigs made up 24.4%, cattle accounted for 14.0%, goats for 8.5% and horses for 2.3%. From 1867 to 1872, an average (six-year average) of 28,921 cattle, 385,719 pigs and 45,267 sheep and goats were exported annually. Of the field crops, corn accounted for 55% of the sown area and wheat for 31%, followed by barley, oats, rye, millet and buckwheat. Large quantities of grain, mostly wheat, were exported. Thus, in 1868, 59,000 tons of wheat were exported from Serbia (*Milićević*, 2005).

On the expansion of Serbia's borders after the end of the war with the Ottoman Empire in 1878, Serbia gained four new districts (Niš, Pirot, Vranje and Toplica), in which in 1884 there were 321,772 inhabitants. In the same year, based on inventory data, it was determined that there were 21,355 horses and donkeys, 112,109 cattle, 61,586 pigs, 352,914 sheep and 119,268 goats in these four districts (*Milićević*, 2006).

In addition to livestock, products of animal origin such as meat products, cheese, tallow, wool, leather and horns were also traded. At the time of Prince Milos's rule (mid-1800s), Serbia did not have a foreign trade deficit, because it exported ten times more than it imported (*Vučković*, 2005).

The favourite vegetables of the population of Serbia were cabbage, beans (pulses), onion, tomato, and then potato in the 19th century (from the time of Karadjordje in the early 1800s). Broad beans and lentils were more rarely used in the diet. However, the most important vegetable was bell pepper (capsicum, locally called paprika) (sometimes very hot types).

In all towns in Serbia, huge quantities of bell peppers were a characteristic of markets in the autumn. In summer, bell peppers were eaten raw, and in autumn they were sun-dried in wreaths on the external walls of houses for consumption over the winter. Bell pepper was more a staple vegetable in the diet, not just a spice. Pickles were prepared from assorted vegetables for winter consumption. The traditional types of fruit in Serbia were apples, pears, blackberries, blueberries, strawberries and the red plum named *ranka*. The Ottomans brought peaches, apricots, medlars and some types of plums (*požegače*, for example) to Serbia. Spinach, cauliflower, kale and kohlrabi were unknown in Serbia in the 19th century. Of all the types of fruit in Serbia, the most widespread was the plum, so it was a significant source of income for the local population. Most of the plum products, such as prunes, jams and plum brandies, were intended for export (*Cvijić*, 1991; *Kanic*, 1985a).

Food production in Serbia in the 20th and 21st centuries

Just as agriculture changed the world, it also changed Serbia. The country has also gone through a phase of deforestation with the aim of obtaining a larger arable land area to be used for crop and animal farming, and fruit cultivation including viticulture and olericulture. In the 20th and 21st centuries, this deforestation especially refers to the part of Serbia south of the Sava and Danube rivers. North of these rivers, however, the land has also undergone metamorphosis. There, a network of canals excavated in the 18th and 19th centuries was completed in the 20th century. It drained the land and made the wetland one of the most fertile soils in Europe (*Baltić and Marković*, 2017). In Serbia, agriculture, and some branches especially, depended on differences in geographic relief and climate, soil richness, forms of land ownership, and people's way of life and religion. Livestock breeding has always had a special place in agriculture. In the world, as well as in Serbia, the relationship and importance of certain cultivated animal species and nutritional plants has changed over time (*Cvijić*, 1991; *Milićević*, 2005; *Milićević*, 2006; *Lazarević and Lazarević*, 2016).

There have been changes in pig breeding. As the areas under grain (corn, wheat and barley) or in orchards, vineyards, and vegetable gardens increased at the beginning of the 20th century at the expense the forests, so gradually greater numbers of people switched to feeding pigs on grain (corn in particular). Until the Annexation crisis and the Customs War (1906–1908), the export of live cattle enabled significant prosperity for Serbia. Pork fat was actually very important for Austria-Hungary. In the powerful Austro-Hungarian empire, military service was mandatory and it was necessary to provide sufficient amounts of energy sources in soldiers' diets. The simplest and easiest means was to provide dietary energy by using pork fat. Vegetable oils at that time were rarely used in in people's diets (*Baltić et al*., 2010; *Bošković et al.*, 2015). The Customs War forced Serbia to start developing its own slaughter industry and meat processing. Meat products were exported to England, Italy, Switzerland, France and Algeria. Serbia exported over 9,000 tons of meat products in 1908. For Serbia as a rural country, the primary goal of the rural economy was maintenance of the rural family, which was the basic economic and consumer unit. In 1912, meat production in the regions south of the Sava and Danube rivers was at the level that that part of Serbia has today. Agriculture enabled Serbia to acquire and maintain one of the most modern armed forces in the world before the First World War (*Baltić et al.*, 2010).

At the beginning of the 20th century, agronomy became an increasingly important activity. The land was arable, the inhabitants cultivated it with pleasure, and in good years there was enough food for even the poorest. The fields were, for the most part, under corn but with some wheat. As a rule, corn gives better yields than wheat, the grain is used for human and livestock nutrition, and the stalks for livestock nutrition. Nowadays, the granaries of Serbia are in Vojvodina (the north) and the river valleys. Corn is the most commonly grown grain for livestock feed and for export. Wheat production meets the local need for bread. Other cereals, like barley, oats and rye, are cultivated mostly for livestock nutrition (*Lazarević and Lazarević*, 2016).

In the 20th century, the world intensified agricultural production from decade to decade, even from year to year, which was quite understandable for a century in which the population tripled, i.e., increased from two to six billion. This number of inhabitants has necessitated constant increases in food production. This was achieved thanks to progress in many areas of life. Numerous measures, both in new plant varieties, fertilizers, irrigation and plant protection as well as in livestock management like changes in breed and composition, selection, diet, housing conditions, etc. have progressed the most important branches of agriculture. Serbia did not lag behind the world in that respect either. In arable farming, yields per unit area have increased substantially. Thus, the wheat yield in

1939 was 1,390 kilograms per hectare, while at the end of the 20th century, it was 4,483 kilograms per hectare (*Statistical Yearbook of Serbia and Montenegro*, 2000–2011). The corn yield increased from 1,670 kilograms per hectare in 1939 to 7,900 kilograms per hectare in 2020 (*Statistical Yearbook of RS*, 2021). Part of the land area was sown with silage corn, which accounts for 1% of the total sown area of this grain, with the yield of silage corn per hectare exceeding 20 tons (*Statistical Yearbook of RS*, 2021). The yields of rye, barley, oats, sugar beet, sunflower and soybeans have also increased greatly. There was also a change in the structure of sown field crops. After the Second World War in 1947, close to 37% of arable land was sown with wheat, and 46% with corn. Fifty years later, 30% of arable land was sown with wheat and 51% with corn (*SRS Statistical Yearbook*, 1997). From the total used agricultural land in the Statistical Yearbooks of Serbia, data are kept on agricultural areas, production and yields of grain (corn, wheat), sugar beet, oil seed plants (sunflower, soybean, oilseed rape), vegetables (potatoes, beans, cabbage, kale, bell peppers, tomatoes) fodder plants (alfalfa, clover, pastures, meadows) and fruit (apples, plums, cherries, raspberries, grapes, strawberries).

Food production in the 21st century

The data presented above indicate that the basic goal, i.e., the food security of the country in terms of supply of bread grain (wheat, above all) oilseeds (sunflower, soybean) and sugar beet was ensured towards the 1980s. On the other hand, corn and by-products of soybean, sunflower, barley, wheat and sugar beet were used to feed livestock. A significant part of corn, wheat, and other field crops and their products was intended for export (*Baltić and Marković*, 2017).

From the end of the Second World War until the 1990s, meat production in Serbia constantly grew. In the 1990s, total meat production in Serbia amounted to 626,000 tons (155,000 tons of beef, 291,000 tons of pork, 32,000 tons of sheep meat, 113,000 tons of poultry and 35,000 tons of offal). In the total production in 1990, pork constituted 46.5%, beef made up nearly one quarter, poultry 18.0%, sheep meat 5.0% and offal 5.6% (*Statistical Yearbook of the SRS*, 1991). However, by 2006, total meat production had fallen to 459,000 tonnes. Over that time, the production of beef decreased by 42%, pork by 13%, sheep by 34% and poultry by 42%. Overall, meat production has decreased by 27% since 1990 (*Statistical Yearbook of RS*, 2007). The average annual total meat production for a three-year period (2018–2020) was 518,000 tons, of which an average of 74,000 tons was beef (14.28%), pork averaged 300,000 tons (57.91%), sheep meat 32,330 tons (6.24%) and poultry 111,660 tons (21.56%) (*Statistical Yearbook of RS*, 2021). From these data, it can be concluded that meat production has stagnated, because poultry production has fallen since the 1990s. However, the most worrying decline is in beef production. Beef meat is the most sought after on the world and European Union (EU) markets, and Serbia has been exporting ever decreasing amounts in recent years. The Serbian market is supplied annually (average 2018–2020) with 2,187 tons of fish, of which 777 tons of fish originate from aquaculture and 141 tons are from open waters. During 2018–2020, the average annual production was 14,990,000 litres of milk, 17,590,000 million eggs and 826 tons of honey (*Statistical Yearbook of RS*, 2021). In Serbia, the 19th and the beginning of the 20th centuries will be remembered for the export of pigs (mainly to Austria-Hungary). The 20th century, and especially since the 1960s, will be remembered for the export of beef to Greece, Italy, Great Britain and Arab countries and pork products (canned ham), to Germany and other European countries, as well as the United States.

The disintegration of Yugoslavia, wars and sanctions put the agricultural production of Serbia in a difficult position. Today, the export of grain (corn and wheat) dominates in Serbia, while the export of livestock products dominates in more developed countries. Production capacities in crop production, and especially in livestock production, were reduced in the last decade of the 20th century. Livestock production accounts for 25% of the total agricultural production in Serbia, but over 50% in EU countries. Surplus corn is exported, even if it would be more economically justifiable to use for cattle feed, especially for beef production, so that Serbia could have enough beef for export. Data on the global production of meat and grain (corn and wheat) in 2011 show an anticipated increase in production will be needed until 2030 and 2050, respectively, when the world population should be around eight to nine billion. World meat production in 2011 was 269 million tons. The needs in 2030 will be 388 million tons (1.44-fold increase), and in 2050, 460 million tons (1.71-fold increase). Corn and wheat production in 2011 was 1.585 million tons, of which 952 million tons (60%) were intended for human consumption and 635 million tons (40%) for animal nutrition. In 2050, the total production needs will be increased 1.52-fold, animal nutrition needs by 1.71-fold, and human nutrition needs by 1.39-fold. The average

annual consumption of meat per capita in the world in 2011 was 39 kg, and ranged from 3 kg in Bangladesh and India to 116 kg in the United States. From 2000 to 2011, meat production increased 1.3-fold (*Alexandratos and Bruinsma*, 2012).

Sustainable development and organic production

Organic production is a part of agricultural production based on ecological principles of sustainable development. It includes primary food production, food processing and distribution. According to projections, food security and organic production could be achieved by 2050, but arable land would have to increase by 16% to 30%, depending on climate change. If climate change were more expressed, the increase in arable land would have to be greater. However, organic production would not significantly affect the consumption of irrigation water and or the emission of damaging gases. Organic production would increase deforestation and increase soil erosion, but would reduce energy consumption, drastically reduce the amount of pesticides and nitrogen in the environment (water, soil), and would reduce the quantity of livestock and meat produced. Projections to increase the production of organic food differed from country to country. It is being considered from the angle of a complete transition to organic production, to systems in which part of conventional production (say, 20 or 50%) would be replaced by organic production. In all cases, the advantage of the more nutritionally valuable food provided by organic production is emphasized.

With 7–8 million inhabitants, Serbia could produce food for 40 million people with better use of its already available arable land, enlargement of agricultural land holdings, application of modern biotechnical measures, irrigation etc. (*Baltić and Marković*, 2017). Today, 424,000 hectares (12.5%) of arable land are uncultivated in Serbia, which is more than the average area under pastures or fodders, i.e. meadows, clover, alfalfa, plus soybean and sunflower. Only the areas under wheat and corn are larger than the area of uncultivated but available arable land in Serbia (*Statistical Yearbook of RS*, 2021). If the uncultivated areas were used as meadows with an average yield of two tons per hectare, 848,000 tons of quality fodder plants for livestock feed would be obtained. These lands are mostly uncultivated pastures and abandoned orchards overgrown with weeds. These uncultivated areas could be converted very quickly into organic production because they have not been used for years, and they are not particularly burdened with environmental or agrochemical pollutants. This measure would increase the area in organic production in Serbia by 50- to 60-fold.

Food security encompasses availability (accessibility to the population), accessibility (depends on purchasing power), stability (possibility of procurement at any time), energy, nutritional value, meeting water needs in production (especially irrigation water), land degradation, climate change, plant and animal diseases, political relations (sanctions, wars), population growth, energy consumption (for land cultivation and application of agro-technical measures), homogenization of consumption (excessive consumption of the same type of food), price formation (supply and demand) and reducing the amount of food wasted (*Pérez-Escamilla and Segall-Corrêa*, 2008; *Maxwell,* 1996; *Godfray et al.*, 2010). International organizations such as the United Nations (UN), the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) consider and promote food security, wise food consumption and food needs for the entire world population (*Baltić and Marković*, 2017).

Today, plant production is largely based on advances in molecular genetics, genetic engineering techniques and the creation of genetically modified varieties of corn, soybean, rice and other plant species to have other improved characteristics in addition to high yields (disease resistance, tolerance to high temperatures etc.) Genetically modified plant species are grown in the United States, Canada, China, Japan and some African, South American and EU countries. The cultivation of GMO plants is still not allowed in Serbia, but these plants are at ourante portas. Discussions among scientists about the harmfulness of GMOs to human health are numerous and very often contradictory (*Basu et al.*, 2010; *Taheripour et al.*, 2016; *Hilbeck*, 2015).

New possibilities in food security and production

Today, in the world, the development of alternative food sources using other means apart from traditional agriculture is underway. This primarily refers to *in vitro* meat production. Research in this field has intensified in recent years and is attracting the attention of a growing number of scientists (*Baltić et al.*, 2013). The use of insects as animal feed and human food is also under development. Also, microengineering has been used to cultivate algae that are rich in proteins and unsaturated fat-

ty acids. At the end of the production process, a fine, edible, green powder was obtained that could provide significant amounts of protein for human consumption (*Specht et al.,* 2017).

Today, there is increasing concern about the world-wide problem of food waste, which significantly contributes to food insecurity. Overall, 1/3 of food produced is not used, i.e., it is discarded. In the cereal production chain, 20% is lost during harvesting and sorting, 3% during delivery and storage, 2% during processing, 9% in wholesale and retail and 19% in households. According to FAO data, 1.3 billion tons of food are thrown away in the world every year, which is enough to supply food to the world's malnourished population. Losses in the food production chain can be reduced, depending on numerous factors, such as appropriate mechanization, better training of workers, protection against pests, and proper storage conditions. In recent years, there has been a focus on the unnecessary discarding of food, especially in households. Most often, bread and fruits are discarded, while there is less squandering of meat and meat products. In Serbia every year, about 250,000 tons of food are thrown away. Everywhere in the world, including in Serbia, food waste is most common in urban areas. In rural areas, unused food is very often used for animal feed, particularly for pigs and poultry (*Janjić et al.*, 2019).

Conclusion

All periods in the development of Serbia, from the Slavs' arrival on the Balkan Peninsula until today, have ensured the food security of the population with appropriate local agricultural production. There were famine years during early wars, during the rule by the Ottoman Empire and during natural disasters too, but not the mass starvation and deaths that have been recorded, and unfortunately, still exist in other parts of the world. However, since the 1990s, when Yugoslavia disintegrated and Serbia experienced its latest wars and sanctions, there has been a crisis in agricultural production. The reasons for the crisis are numerous and complex. Our intention is not to discuss them in detail in this paper, because it is the concern of experts from different fields, knowledge profiles, experiences, views and opinions. Certainly, Serbia can produce far more food than it needs, just as it can certainly throw away (lose) less in the entire chain of food production, trade and distribution. Serbia could significantly increase its production of organic food and be an example in supporting the concept of corporate social responsibility and the concept of sustainable development. We should get to know our country from the past from numerous documents and relevant testimonies written by historians and travel writers. How would Cvijić, Kanic, Jiriček, Pirh, Lamartin and Čelebi describe today's Serbia?

Proizvodnja i sigurnosti hrane na području Srbije: istorijski kontekst i sadašnji trenutak

Vesna Đorđević, Milorad Mirilović, Nenad Katanić, Jelena Janjić, Drago Nedić, Danijela Šarčević, Baltić Ž. Milan

A p s t a r k t: Cilj rada je da ukaže na značaj izvora i proizvodnje hrane od vremena kada je čovek postao sakupljač plodova i lovac, zatim i poljoprivrednik, sve do današnjeg vremena kada se sve više govori o sigurnosti hrane — „food security" sa aspekta obezbeđenja dovoljnih količina hrane za sadašnje i buduće generacije. U radu je predstavljena proizvodnja hrane u Srbiji od dolaska slovenskih plemena na Balkansko poluostrvo do savremenog doba, sa ciljem da se ukaže na istorijski razvoj proizvodnje hrane i neophodnost društveno odgovornog ponašanja kako bi se sačuvali resursi koje imamo za buduće generacije.

Ključne reči: *Srbija, hrana, poljoprivreda, prošlost, budućnost.*

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Review paper

Food allergens — food safety hazard

Jovanka Popov Raljić[1], Milica Aleksić[2], Vesna Janković[3]

A b s t r a c t: Food allergens have appeared in the last two decades as a concealed form of threat which significantly endangers public health, and their labelling on food products, drinks, and non pre-packed gastro-products is clearly defined by legal regulations. Food allergy is a life-threatening chronic condition that substantially impairs quality of life. Food allergies constitute a significant public health problem that affects children and adults and is a considerable burden on health, medical systems and emerging economies. Appropriately managing food allergies has become an issue for the food industry because of the rising number of individuals with food allergies.

Keywords: *food allergy, food allergens, big eight.*

Introduction

Food allergy is a life-threatening chronic illness that severely limits the individual's quality of life. These allergies are a significant part of public health policy. Children and adults are afflicted, and the cost to health, medical systems, and expanding economies is enormous (*Greenhawt*, 2016). Food allergy is described as a negative health consequence caused by a specific immune-mediated reaction that happens consistently after eating a specific food (*Boyce et al*., 2010; *Wang and Sampson*, 2011), and food-specific IgE antibodies, cellular processes, or both can be involved (*Muraro et al*., 2014).

Food allergy is defined by the European Academy of Allergology and Clinical Immunology (EAACI) as a subclass of adverse reactions in which the immune system plays a role. Food allergies are categorised as IgE-mediated, non-IgE-mediated, or

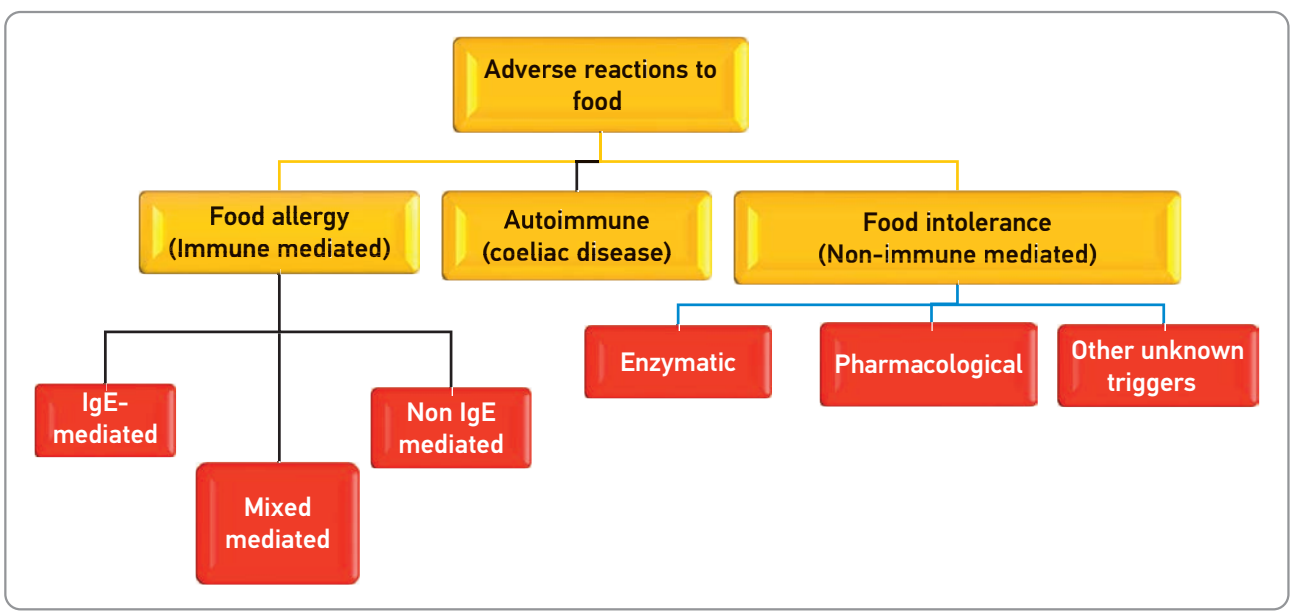

Figure 1. Classification of adverse reactions to food (*Johansson et al.*, 2001; *EFSA*, 2014)

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mixed IgE and non-IgE-mediated reactions based on the mechanism of occurrence (*Sampson* 2004; 2007; *Wang and Sampson*, 2011).

According to the European Food Safety Authority (*EFSA*, 2014), adverse food reactions are divided into classes based on the pathogenic mechanism (Figure 1). They include immunologically mediated reactions, which are mediated either by IgE antibodies, by cells (non-IgE-mediated) or both (mixed), and non-immunological responses (food intolerance), which are dependent on enzyme deficiencies or pharmacological reactions or, in the majority of cases, arise by unknown mechanisms (*Vickery et al*., 2011; *Muraro et al*., 2014; *Waserman and Watson*, 2018).

Food-induced immune-mediated adverse reactions show as clinical signs and symptoms of varying intensity and duration, affecting many organs and systems. Food-induced anaphylactic responses are mediated by IgE and can occur at any age (*Yue et al.*, 2018). Protein-induced enterocolitis and eosinophilic oesophagitis are two illnesses caused by non-IgE-mediated food allergies (*EFSA*, 2014; *Connors et al.,* 2018).

Food allergen epidemiology

In developed countries, the prevalence of food allergies is unknown. The major causes for this uncertainty are the scarcity of studies available for specific geographic areas and the use of diverse procedures among studies to derive prevalence data (*Huiwen and Leung*, 2018).

Food allergy prevalences and patterns are significantly diverse in different parts of the world (the second wave of the allergy epidemic). A complex combination of genetic, epigenetic, and environmental variables could create differences in food allergy epidemiology (migration, climate and infant feeding practices). When considering data from Europe, based on a meta-analysis of published food challenge data, cow's milk, tree nuts, soy, hen's egg, peanut, wheat, fish and shellfish were the most common elicitors of food allergy in Europe with an estimated accumulated prevalence of 2.1% (1.2 to 3.3%) of the population, while in the United States, and Australia/New Zealand, the prevalence of food allergy has been estimated to be around 3% (*Prescott and Allen*, 2011; *Holzhauser et al*., 2020). However, there was a lot of variation in the research utilized to determine the prevalence of food allergies. There is inadequate objective evidence to draw any conclusions on time patterns in the prevalence of food allergies in Europe. Egg, peanut, cow's milk, seafood and other nuts cause over 75% of allergy reactions in youngsters. Adults are allergic to around half of the fruits and vegetables in the Apiaceae family, as well as numerous nuts and peanuts (*EFSA*, 2014).

Food allergy prevalence varies by region according to environmental (e.g. pollen exposure or food habits) and individual factors. Individual characteristics considered crucial in the development of food allergy include sex, age, family history of atopy and the presence of other allergic illnesses. Due to variances in genetic background, exposure to offending foods, and eating habits, extrapolating prevalence data on specific food allergies from a single European country to the entire European population has limited accuracy.

There is currently no available treatment for food allergies. To avoid an allergic reaction, which can be severe and life-threatening, strict allergen avoidance remains the most effective allergy management strategy (*Muraro et al.*, 2014). Identification of the relevant dietary allergen is essential for successful allergy avoidance. The European Union (EU) food information regulation was created to allow consumers to easily identify relevant allergies (*Regulation EU* 1169/2011, 2011), and in Serbia, the *Rulebook on declaration, labeling and advertising of food* (*Official Gazette of RS*, No. 19/2017, 16/2018, 17/2020 and 118/2020), *Rulebook on health correctness of diet foods* (Official Gazette of RS, No. 45/2010, 27/2011, 50/2012, 21/2015, 75/2015, 7/2017 and 103/2018 — other rulebook) requires the mandatory labelling of 14 allergenic foods or food groups, including the above-mentioned allergens, when they are used as ingredients in manufactured foods.

Food allergens

The EU food information regulation (*EU*, 2011) does not apply to non-ingredient allergenic components that may arise via cross-contact, such as during manufacturing or packing. Food makers can use voluntary precautionary allergen labelling (PAL) to educate and protect allergy sufferers in the event of the presence of unintended allergens (*Muraro et al*., 2014a). Unfortunately, mislabelling and sporadic use of PAL that is not based on quantitative allergy risk assessment might result in a mismatch between labelling and allergen presence (*Allen et al*., 2014; *Crotty and Taylor*, 2010; *Remington et al*., 2015), and as a result, allergic consumers face considerable risks, and their product options are limited

(*Holzhauser et al*., 2020). In Serbia, the Ordinance on declaring, labeling and advertising of food (Official Gazette of RS, No. 19/2017, 16/2018, 17/2020 and 118/2020) and the Ordinance on the health safety of dietary foods (Official Gazette of RS, No. 45/2010, 27/2011, 50/2012, 21/2015, 75/2015, 7/2017 and 103/2018) provide allergen legislation considering 14 food ingredients that can cause allergic reactions or intolerance, method of declaration, recommended method for detection of gluten in foods, etc. In this paper, data for the eight major allergenic foods will be presented (the big eight).

Cereals containing gluten and cereal products

Wheat flour is used as a raw material for bakery items (bread, pastries, pizza), pasta (noodles, pasta, spaghetti), some confectionery (cakes, biscuits, gingerbread), and ready meals (cream soups, sauces etc.) (*Psodorov*, 2014; *Popov-Raljić*, 2016).

In order to generate a functional product, flour formed by grinding pseudocereals such as amaranth, buckwheat, quinoa, sorghum or other plant material is increasingly utilized in addition to wheatflour, as are combinations of different types of flours from small grains such as oats, barley, rye or corn (*Alvarez-Jubete et al.,* 2010; *Sakač et al.,* 2011).

Table 1 illustrates gluten-containing cereals (wheat, rye, oats, barley, spelt, kamut, and cross-bred types) and their reference doses in relation to some of the most often consumed foods (*Popov-Raljić et al.,* 2017). The majority of gluten-free items on the market are made with starch; however, gluten-free food makers frequently employ whole grains like corn, corn, amaranth and quinoa, which are high in fibre, iron and vitamin B (*Lorenzo et al.,* 2018).

Allergic reactions to wheat and other cereals are most frequent in infants, and they normally pass during the first few years of life. IgE-mediated cereal allergy symptoms range from minor local skin or gastrointestinal reactions to more acute, often life-threatening anaphylactic episodes. Bakers' asthma (occupational exposure to grain flour dust) and, less commonly, IgE-mediated allergy

Table 1. Cereals containing gluten and cereal products — *Popov-Raljić et al.* (2017), *The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/*(2020)

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
Wheat, wheatberries, rye, barley, spelt, kamut®, triticale, durum wheat or semolina, club wheat, emmer, einkorn, farro, and varieties created by their crossing, except glucose syrup based on wheat and dextrose, maltodextrin based on wheat, glucose syrups based on barley, and cereal distillates or ethyl alcohol of agricultural origin for the manufacturing of strong alcohol beverages obtained from cereals.  Reference dose = 0.7 mg protein **(VITAL 3.0, 2019, ED_{01})**	Celiac disease or gluten intolerance. Cereal allergens can cross-react with pollen allergens.	✓ Flour ✓ Starch ✓ Bran ✓ Rusks ✓ Bread, bread ✓ Grits ✓ Couscous ✓ Hydrolysed vegetable protein (if derived from wheat)

Table 2. Allergenic proteins (*Fagopyrum esculentum* — Common buckwheat, *Fagopyrum tataricum* – Tartarian buckwheat, *Triticum aestivum* — wheat, *Triticum turgidum ssp durum* — Durum wheat) (WHO/IUIS — Allergen Nomenclature Sub-committee, *www. allergen. org-allergen nomenclature*)

Fagopyrum esculentum	**Allergen exposure route**	
Allergen	Food	
Fag e	Fag e 2–5	
Fagopyrum tataricum		
Allergen	Food	
Fag t	Fag t2, Fag t6	
Triticum aestivum	Food	Airway
Allergen		
Tri a	Tri a 12, 14, 17- 21, 25–28, 36, 37, 41–45	Tri a15, 29–35, 39, 40
Triticum turgidum ssp. durum	Food	
Tri tu	Tri tu 14	

related to exercise, known as wheat-dependent exercise-induced anaphylaxis, are examples of wheat allergy. Rice is generally tolerated by people sensitive to wheat-related crops (barley, oats, and rye). Rice allergies are uncommon in Europe and America, but they may be more common in Asia. Gluten-related illnesses have gained increased epidemiological significance, with an estimated global frequency of roughly 5%. Gluten-related illnesses include celiac disease, wheat allergy, and non-celiac gluten sensitivity. Gluten-derived peptides cause a T-cell mediated autoimmune reaction in celiac disease (*Rubio-Tapia and Murray*, 2010). The classical enteropathy and malabsorption syndrome are caused by an autoimmune inflammatory cascade in the small bowel.

Celiac disease is the most well-known gluten-related condition to date: the genetic propensity of patients, the relationship with other autoimmune disorders and celiac disease's consequences have all been widely researched. Wheat allergy is an allergic reaction to proteins found in wheat and similar cereals, with varying clinical manifestations depending on the route of exposure. In this condition, Immunoglobulin E (IgE) antibodies mediate the inflammatory response to a variety of allergenic proteins (alpha-amylase/trypsin inhibitor, non-specific lipid transfer protein (nsLTP), gliadins, and HMW glutenins) (*Ludvigsson et al.*, 2013). Non-celiac gluten sensitivity is a third form of clinical reaction to gluten consumption. In the absence of celiac disease or wheat allergy, patients with non-celiac gluten sensitivity often have a wide spectrum of intestinal and extraintestinal symptoms quickly after consuming gluten-containing foods (wheat allergy) (*Catassi et al*. (2013); *Elli et al.,* (2015). Many allergenic proteins are involved in wheat allergy and the latest updated version of the WHO/IUIS Allergen Nomenclature Database describes many different well-classified wheat allergens (Table 2).

Eggs and egg products

Eggs and egg components/ingredients are frequently widely used in a variety of culinary products, including bakery and confectionery, gourmet (soups, sauces, dressings) and meat products. According to the USDA, the edible portion of the egg is made up of 63% egg white and 27.5% yolk, while 9.5% is egg shell, including the shell membrane. Egg is one of the most common allergenic foods, with an allergy prevalence of 1.8 to 2% in children under the age of five (*Lee*, 2017, *Loh and Tang*, 2018). Table 3 shows general allergy information for eggs and egg products.

Egg white contains the majority of the proteins linked to egg hypersensitivity (*Réhault-Godbert et al.,* 2019). According to *Dumont and Delahaut* (2010), both egg white and egg yolk contain clinically significant allergic egg proteins. Table 4 shows the molecular and biological properties of the identified egg allergens.

Table 3. Eggs and egg products — *Popov-Raljić et al.* (2017), *The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/*(2020)

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
 Reference dose = 0.2 mg protein **(VITAL 3.0, 2019, ED_{01})**	Egg allergy is prevalent in youngsters, but by the age of three, more than half of them have outgrown it. Individuals may get anaphylactic crises.	✓ Powdered eggs, dried eggs or pasteurized eggs ✓ Albumin ✓ Egg glaze ✓ Mayonnaise ✓ Note: lysozyme (produced from egg albumin), which is used in refining wines, has a low risk of producing responses. However, when lysozyme is utilized for other purposes (for example, as a cheese preservative), it might have negative consequences health consequences (Schneider and Pischetsrieder, 2013)

Table 4. Molecular and biological properties of identified egg allergens *Dumont and Delahaut* (2010); *Sakai and Teshima* (2015)

EGG WHITE PROTEINS	
Ovomucoid (Gal d 1)	Inhibitor of trypsin activity Antimicrobial properties Activation of the immune system Antioxidant properties Bacteriolytic activity Antiviral activity
Ovoalbumin (Gal d 2)	
Ovotransferrin (Gal d 3)	
Lysozyme C (Gal d 4)	
EGG YOLK PROTEINS	
Serum albumin — α-livetin (Gal d5)	Inhibitor of trypsin activity Antimicrobial properties Activation of the immune system Antioxidant properties Bacteriolytic activity Antiviral activity
YGP42 (Gal d6)	
Myosin light chain 1f (Gal d7)	
α-parvalbumin (Gal d8)	
ß-enolase (Gal d9)	
Aldolase (Gal d10)	
OTHER EGG YOLK ALLERGENS These minor allergens have not been designated by the WHO/IUIS Allergen Nomenclature Sub-Committee.	
Phosvitin	This is a highly phosphorylated molecule with a high capacity for cation chelation. More than 90% of the iron in an egg is bonded to phosvitin in the yolk, according to estimates. Phosvitin has antibacterial and antioxidant properties as a result of this characteristic (*Sakai and Teshima*, 2015)
Apovitellin	The other egg yolk allergens are apovitellenin-containing lipoproteins. Apovitellenins I (Gal d Apo I) and VI (Gal d Apo VI) have been reported to show IgE-binding activity (*Sakai and Teshima*, 2015)

Table 5. Clinical symptoms associated with egg allergy — *Lack,* (2008)

Presentation	Population	Clinical Manifestation	Natural History	Egg Component	Major Allergens
Egg-white allergy	Atopy and eczema patients, in particular, are young children.	After intake, contact urticaria and systemic type 1 hypersensitivity symptoms occur.	Resolves by 7 years of age	Egg white	Ovomucoid, ovalbumin
Bird-egg syndrome*	Adults who have been exposed to birds, primarily women	Type 1 hypersensitivity symptoms after consuming egg yolks; respiratory problems following exposure to bird feathers	Persistent	Egg yolk	α-livetin (chicken serum albumin), cross-reactivity with bird feathers
Occupational egg allergy ("egg-egg" syndrome)	Adults working in the food and confectionery industries	Variable type 1 hypersensitivity symptoms (usually minor) after consuming egg white; respiratory symptoms following exposure to aerosolized egg white	Persistent	Egg white	Ovalbumin, ovomucoid, conalbumin, lysozyme

a * Oral symptoms have been reported with hen's eggs, although respiratory problems have been reported with exposure to a variety of birds

Eggs frequently cause food allergy with symptoms from a slight rash to anaphylaxis. The prevalence is expected to range from 0.2% to 7% (*Rona et al.,* 2007; *Lee*, 2017). Due to differences in patient sensitivity and the specificity of the allergen, the amount of food allergen required to provoke an allergic reaction is rarely known with any accuracy. *Taylor et al*. (2002) consider that cumulative doses that cause allergies range from 0.13 mg of raw whole egg to 200 mg of dry protein from whole egg.

The egg contains many biologically active components (*Réhault-Godbert* et al., 2019). Because egg components have many purposes (e.g., lysozyme serves as a preservative, lecithin serves as an emulsifier, and provitamin A serves as a colorant), allergens can be found in any of these food technology products if they are egg-based or egg-derived (*Audicana Berasategui et al.,* 2011).

Egg allergy is most common in children under the age of two (*Boyano-Martínez et al.,* 2002), and according to the same author, 66% of children with allergies outgrow their egg allergy after the fifth year (*Savage et al*., 2007). According to (*Caubet and Wang,* 2011) one study, children become largely immune to egg allergy at a later age (6 years old), with 37% at 10 years old and 68% at 16 years old recovering from their childhood allergy. However, over half of their 12-year-old patients were unable to consume concentrated eggs. The fact that the ratio decreases with age suggests that by the time they reach school age, newborns and toddlers have developed resistance and tolerance to eggs.

Milk and milk products

Milk is considered a complete food since it contains essential proteins, minerals, lipids and carbohydrates for human health (*Pereira et al.*, 2012). Milk includes high-quality proteins, fats, vitamins and minerals (such as potassium, phosphorus and calcium), but despite its nutritional benefits and widespread recommendations, milk consumption in Western countries is rapidly declining (*Lucarini,* 2017; *Chalupa-Krebzdak et al*., 2018; *Silva et al*., 2020). Cow's milk allergy is a common diagnosis in babies and children, and it usually disappears by the age of six. It manifests as an allergic reaction, which is the immune system's reaction to a specific milk protein. (*Edwards and Younus*, 2021).

Table 6 presents the basic characteristics, distribution and individual food/gastronomic products in which milk and dairy products can be found.

According to a survey of the literature conducted by the University of Portsmouth, forty reports on allergic reactions to milk and cow's milk products were published between 1982 and 2012. By far the most common food allergy is milk allergy (*EFSA*, 2014) which can be classified according to IgE and non-IgE mediated symptoms (*University of Portsmouth*, 2013).

Table 6. Milk and milk products — *Popov-Raljić et al.* (2017)
The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/(2020)

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
Milk and milk products including lactose Exceptions are: whey when it is used to make distillates or agricultural ethyl alcohol for strong alcoholic and alcoholic beverages; lactitol.  Reference dose = 0.2 mg protein **(VITAL 3.0, 2019, ED_{01})**	Cow's milk allergy is the most prevalent allergy in young children, affecting 2–7% of infants under the age of one year. By the age of three, around 87% of children have outgrown their allergy. Cow's milk has a high level of cross-reactivity with the milk of other mammals like sheep, goats, and buffalo.	✓ Whey ✓ Casein ✓ Milk powder ✓ Lactose ✓ Butter, cheese, creams, yogurt, butter

Lactose is ingested and hydrolysed by lactase, an enzyme found in the microvillus membrane of enterocytes, into glucose and galactose, which are then absorbed. Undigested lactose can cause lactose intolerance symptoms if lactase activity is insufficient or absent. Subjects with galactosaemia, an inherited abnormality of galactose metabolism, also do not "tolerate" lactose, although their symptoms are more severe and differ significantly from those of lactose-intolerant subjects. Lactase activity can be stable, low, or absent due to a change (in newborns) or a drop (in adults) in lactase gene expression (primary lactase deficiency). Secondary lactase insufficiency can be caused by intestinal disease processes that destroy the epithelium of the small intestine. This condition is reversible once the underlying sickness is treated. Lactase-nonpersistence is a genetically determined and normal developmental phenomenon characterized by the down-regulation of lactase activity that occurs soon after weaning in most ethnic groups. Lactose intolerance affects people of all ages due to a deficiency in the enzyme lactase, which results in poor lactose digestion and, as a result, symptoms such as bloating, abdominal pain, and diarrhoea after ingesting milk and dairy products (*Silva et al.,* 2020). Allergenic cow's milk proteins are listed in Table 7.

Table 7. Allergenic proteins in cow's milk (*Bos domesticus*) — *www. allergen. org-allergen nomenclature*

Allergen	Biochemical name
Whey protein	
Bos d2	Lipocalin
Bos d3	S100 calcium-binding protein A7
Bos d4	α-lactalbumin
Bos d5	ß-lactoglobulin
Bos d6	Serum albumin
Bos d7	Immunoglobulin
Caseins	
Bos d8	Caseins
Bos d9	αS1-casein
Bos d10	αS2-casein
Bos d11	ß-casein
Bos d12	κ-casein
Bos d13	Myosin light chain

Many proteins in cow's milk are antigenic and capable of eliciting immunological responses, and sensitivity to diverse cow's milk proteins has been found to be widespread. The most abundant proteins in cow's milk, especially lactoglobulins, caseins, and α-lactalbumin (ALA), are the major allergens, according to studies conducted on large populations of allergic patients; however, proteins present in low quantities, such as bovine serum albumin, lactoferrin and immunoglobulins, have also proved to be important in inducing milk allergies.

Fish, crustaceans, molluscs and their products

Seafood is crucial for human nutrition, health, and economics, yet it can cause major IgE antibody-mediated adverse responses in vulnerable individuals. Fish (cod, salmon and tuna), shellfish (shrimp, crab and lobster), and molluscs (squid, shellfish and snails) are all examples of seafood. Seafood can induce severe acute hypersensitivity reactions, including deadly anaphylaxis (*Sharp and Lopata*, 2014). There are about 20,000 edible fish species, although the most regularly consumed belong to only a few groups (*Actinopterygii*). People who are allergic to fish are generally allergic to a variety of species, and therefore, they should avoid eating all fish.

Adverse reactions to seafood can be immune, such as IgE allergy mediated by the antibody for which the trigger is consumed, or non-immunological, such as poisons or pathogenic elements (*Freidl et al.,* 2017). Fish allergy affects 0.3% of the world's population, while shellfish allergy affects 0.6% (*Sicherer,* 2011), but can reach up to 8% among fish processing workers (*Sharp and Lopata*, 2014). In a survey of 17,280 adults aged 20 to 44 in countries that defined allergy or intolerance to different types of food, and based on reports that food "almost always" causes "illness or discomfort", 2.8% of respondents reported shrimp as a problem, 2.3% oysters, and 2.2% stated they were allergic or intolerant to fish (*Woods et al.,* 2001).

Parvalbumin, the most common fish allergen, as well as a few lesser-known allergens, were studied. Parvalbumins are classified as one of two isoform lineages and both are commonly found in fish (*Sharp and Lopata* 2014), while tropomyosin is common in shellfish (*Chinnappan et al.,* 2020). The cross-allergic reaction to fish and shellfish is high but variable (*Wang et al.,* 2020). With 90% of fish allergy patients reacting to parvalbumin, it is the most common clinical cross-reactive fish allergen (*Lim et al.,* 2008).

The allergenicity of parvalbumin has been studied in a number of fish species and as of 2012, the allergome database (www.allergome.org) (Table 8) has 218 allergenic isoforms of fish parvalbumin listed, while only 27 of these isoforms are actually registered with the World Health Organization (WHO) or International Union of Immunological Societies (IUIS) (*Sharp and Lopata* 2014). For ingestion-related sensitization, a variety of fish allergens have been isolated and identified, but the fish proteins in aerosol relevant for allergic sensitization have yet to be fully defined. Other fish allergens, such as the hormone vitellogenin from Beluga caviar, have been identified in addition to parvalbumin (*Escudero et*

Table 8. Sources of allergens (fish, crustaceans and molluscs) and identified allergens Handbook of Food Allergen Detection and Control, (2015) — *www. allergen. org-allergen nomenclature*

Sources of allergens	Common name	Scientific name	Allergen
Fish	Baltic cod Mackerel Atlantic salmon	*Gadus callarias* *Scomber japonicas* *Salmo salar*	Gad c 1 Sco j 1 Sal s 1–9
Crustaceans	Brown shrimp Tiger shrimp American lobster Chinese lobster Red crab	*Penaeus aztecus* *Penaeus monodon* *Homarus americanus* *Panulirus stimpsoni* *Charybdis feriatus*	Pen a 1 Pen m 1,2,3,4,6,8 and 13 Hom a 1, 3 and 6 Pan s 1 Char f 1
Molluscs	Mussels Noble scallop Abalone Pacific oyster Squid	*Perna viridis* *Chlamys nobilis* *Haliotis midae* *Crassostrea gigas* *Todarodes pacifi cus*	Per v 1 Chl n 1 Hal m 1 Cra g 10101, 10102 Tod p 1

al., 2007) and collagen and gelatine isolated from skin (*Perez et al.,* 2008) and muscle tissues of fish (*Sakaguchi et al.,* 2000). The second major allergen seems to be tropomyosin (*Chinnappan et al.,* 2020; *Lopata et al.,* 2016). Other allergens, such as 40 kDa arginine kinase, which could be a new class of pan-allergens in invertebrates and the skin of some fish species (*Hamada et al*. 2003), have been found and characterized in malignancies in addition to tropomyosin (*García-Orozcoet al.,* 2007).

It is important to note that tropomyosin is not just an allergen in crustaceans; it has also been found in a variety of mollusc species, including mussels, oysters, squid and sticklebacks, making them key food allergens in the exposed population. Molluscs also contain allergens such as big chain myosin, haemocyanin, and amylase, in addition to tropomyosin (*Jin et al.,* 2015).

Soybeans and soybean products

Soybeans (also called soy and soya) cultivated under particular conditions are included in the functional food list, as are other legumes, since they are a good source of biologically active chemicals that can have a beneficial effect (*Popov-Raljić*, 2016). The prevalence of soybean allergy is lower than that of each of the other seven major allergens, which has been used to suggest that soybean could be removed from the Big 8 without causing public harm (*Messina and Venter*, 2020). Soybean protein allergy was the least common allergy in four adult surveys, while milk/dairy and shellfish allergies were the most common. Soybean allergy was found in 0.1% of the population and 0.6% of the population. Soybean allergy was twice as common (0.5%) and similar to wheat allergy in the US NIAID-Children study, while milk/dairy allergy was still 3.8 times more common than soy allergy. Finally, the prevalence of soybean allergy in Canada (0.32%) was in the middle of that found in the two US surveys (*Gupta et al.,* 2019). In general, children with soy food sensitivities tend to outgrow their allergies).

The main allergens in soybean are: gly m 1–8, hydrophobic protein from soybean, defensin, profilin, pathogenesis-related protein, pr-10, bet v 1 family member, beta-conglycinin (vicilin, 7s globulin), glycinin (legumin, 11s globulin), seed biotinylated protein and 2s albumin. Gly m 4 is a major soy allergen, followed by Gly m 5 and Gly m 6 (*Holzhauser et al.,* 2009).

According to EFSA research (*EFSA*, 2014), people who are allergic to peanuts had a greater rate of anaphylactic reactions to soybean protein. In a randomly selected population in Europe, the prevalence of clinically diagnosed soybean allergy was minimal. For pollen allergen Bet 1 v and bovine

Table 9. Soybean and soybean-based products — *Popov-Raljić et al.* (2017), *The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/* (2020)

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
Natural blends of tocopherol (E 306), natural D-alpha tocopherol, D-alpha tocopherol acetate, D-alpha tocopherol succinate, isolated phytosterols and phytosterol esters from soybean oil, in addition to fully refined soybean oil and fats  Reference dose = 0.5 mg protein **(VITAL 3.0, 2019, ED_{01})**	Children are more likely to have a soybean allergy, but they usually outgrow it by the age of two. This allergy can sometimes affect adults. Anaphylactic reactions are extremely infrequent, and most symptoms are mild. There is a possibility of allergenic cross-reactivity between soybeans and other legumes, particularly peanuts, and there have been cases of soybean and cow's milk cross-reactivity.	✓ Soybean flour ✓ Soybean tofu ✓ Soybean protein isolates ✓ Soybean protein concentrate ✓ Soybean formula for infants ✓ Soy sauce

Table 10. Foods that may contain soybean and labels that may indicate the presence of soybean in food
Steinman (1996), Aleksic and Popov Raljić (2015)

Foods that may contain soybean	Labels that may indicate the presence of soybean in food
Baby food	Arabic gum
Pastry	Protein emulsifier, crude soy lecithin
Pudding	Hydrolysed vegetable protein
High protein bread	Lecithin, a protein emulsifier
Minced meat	Soy flour, protein emulsifiers, nutritional supplements
Sausages	Monosodium glutamate
Dehydrated and canned soups	Protein supplement
Chocolate	Soy lecithin
Cakes and biscuits	Soy protein, crude soy lecithin
Oils	Soy protein isolated or concentrated
Hot dog	Soy sauce
Ice cream	Stabilizer
Liquid meal replacements	Starch
Salad dressing	Stabilizer
Sauce (Soy sauce, Worcestershire sauce sweet and sour sauce, tamari sauce etc.)	Tofu
Tofu	Vegetable starch

casein, serological and clinical cross-reactions between soybean and other legumes have been documented.

Table 9 shows a summary of the basic allergenic data for soybean and soybean products (prevalence and severity, as well as foods in which soybean can be found).

Soybean protein products are divided into three categories: additives (soybean flour, semolina, concentrate, protein isolate and hydrolysate), traditional products (soy sauce, miso, tempeh, natto, fermented tofu, unfermented tofu, soy milk and various beverages), and textured soybean products (textured snacks, chunks, structured meat analogues, protein fibre and meat analogues with high moisture content) (*Popov-Raljić*, 2016).

Soybean has a variety of allergenic proteins, with the major proteins glycine and conglycin serving as the primary allergens. Soybean, or its protein lecithin, is often a risk of allergy transmission due to its nearly infinite use in food production. Soybean products are available in a variety of forms to the food industry, including grain, flour, oil, emulsifier, protein supplement and stabilizer. As demonstrated in Table 10, it is typically listed as "hydrolysed vegetable protein" or "lecithin" in the ingredient list.

As a result, because soy is used in a wide range of foods, its declaration and labelling is a major issue. It is commonly found in ready-to-eat foods as a by-product of some of the minor ingredients. For example, if a ready-made/prepared food contains additional margarine, it is noted in the ingredient list, but mention may not be made of the fact that this product (margarine) contains soy or soy derivatives. Soybeans are an important source of oil. Although extracted soybean oil is thought to be safe for consumption, it is possible that a small amount of soy protein will be present (*Aleksić and Popov-Raljić*, 2017).

Peanuts and peanut products

Peanut allergy is a common, long-lasting, and potentially fatal food allergy that is becoming more common in Western countries. One of the most prevalent types of IgE-mediated food responses is peanut allergy (*EFSA*, 2014). It starts early in childhood, is most commonly diagnosed between the ages of 6 and 24 months, is more persistent than milk or egg allergies, and only 20% of patients gain tolerance to peanuts (*Medsen et al*., 2014). Table 11 shows the basic characteristics, distribution, and various food/gourmet goods in which peanuts and peanut products can be found.

So far, 18 peanut allergens have been isolated: Ara h 1 – cupin, Ara h2 – conglutin (2S albumin), Ara h3 – cupin (all three allergens are major peanut allergens), Ara h 4 – renamed as Ara h 3.02, number not available for future submissions, Ara h 5 – profilin, Ara h 6 and Ara h 7 – conglutin, Ara h 8 – pathogenesis–related protein, PR-10 − bet v1 family member, Ara h 9 – nonspecific lipid-transfer protein type 1, Ara h 10, 1, 14 and 15 – oleosins, Ara h 12 and 13 – defensins, Ara h 16 and 17 non-specific lipid transfer protein 2 and 1 and Arah 18 − cyclophilin, peptidyl-prolyl cis-trans isomerase (www. allergen.org/allergen nomenclature).

Because peanuts contain structurally comparable proteins and share common epitopes with other legumes like peas, beans, husks and lentils (*Vereda et al*., 2011), people with peanut allergy demonstrate allergic serological cross-reactivity with other legumes in the family (*Jensen et al.*, 2008).

Table 11. Peanuts and peanut products — *Popov-Raljić et al. (*2017), *The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/* (2020)

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
Peanuts and peanut products  Reference dose = 0.2 mg protein **(VITAL 3.0, 2019, ED_{01})**	A large percentage of people who are allergic to peanuts, as well as other nuts, have an allergenic cross-reaction with other legumes like soy and beans. Heat treatment, particularly roasting, makes peanuts more allergenic.	✓ Unrefined, cold-pressed peanut oil ✓ Peanut butter ✓ Peanut flour ✓ Different peanut protein products ✓ Refined peanut oil

Peanut allergy management now consists of rigorous avoidance of peanut eating and the use of rescue medication in the event of incidental peanut ingestion. However, due to its extensive use as a food ingredient in packaged goods as well as restaurant and catering meals, complete avoidance of peanut is challenging (*Muraro et al*., 2014; *Remington et al*., 2020).

Nuts

Nuts come in a variety of forms, ranging from raw seeds to baked appetizers. In the EU, the average daily consumption of nuts and peanuts was 2.23 g for the entire population. From northern to southern Europe, total nut consumption ranged from 0.61 g per day in Sweden to 4.83 g per day in Spain. The

Table 12. Nuts — *Popov-Raljić et al.* (2017), *Economics of Agriculture/Ekonomika Poljoprivrede The German Federal Institute for Risk Assessment (BfR), https://www.bfr.bund.de/(2020)*

Allergen and reference dose (mg)	Prevalence and severity	Some derivatives (additives) and allergen-containing foods that can cause allergic responses
Almonds, hazelnuts, walnuts, cashew nuts, pecan nuts, Brazil nuts, pistachios, macadamia nuts and their products.  Except for nuts used in distillation and agriculturally produced ethyl alcohol for strong alcoholic and alcoholic beverages. Reference dose = 0.03 mg (walnut) Reference dose = 0.05 mg (cashew nuts) **(VITAL 3.0, 2019, ED_{01})**	Almonds, hazelnuts, walnuts, cashews, pecans, Brazil nuts, pistachios, and macadamia nuts are some of the most popular nuts. Macadamia nuts and their derivatives are a common source of allergies and can induce anaphylaxis responses in persons who are allergic to them.	✓ Nut Butter ✓ Pralines (hazelnuts) ✓ Marzipan ✓ Almond paste ✓ Walnut oils ✓ Worcestershire sauce (some brands contain walnuts)

most popular nuts in Europe are walnuts, almonds, pistachios, and hazelnuts (*Jenab et al*., 2006). This is a prevalent cause of dietary allergies in both infants and adults, and the clinical reaction can be fatal. In the entire UK population, 1.7% of people had a documented allergy to nuts and almonds. Some individuals (around 9%), including some who have had previous severe reactions, outgrow this type of allergy (*Fleischer et al*., 2005). Stone fruit allergies, like fish and peanut allergies, last a lifetime, and most stone fruit allergens are homologous to one another, resulting in frequent cross-reactivity. It is estimated that 20–50% of people allergic to peanuts also have a nut allergy (*Sicherer et al*., 2003).

Table 12 shows the basic characteristics, distribution and individual food/gastronomic products in which nuts can be found.

Walnuts have been proven to be a contributing factor in 1/3 to 1/4 of all food-related anaphylaxis responses (*Cianferoni and Muraro*, 2012). In black walnut (*Juglans nigra*), the most common allergens are jug n1 to n4, vicilin seed storage protein, legumin and 2S albumin seed storage protein, while for walnut (*Juglans regia*), the most common allergens are jug r1 to r8 (*EFSA*, 2014, www. allergen.org/ allergen nomenclature).

Conclusion

Food allergies are a major public health issue. Allergic reactions range from gastrointestinal problems and skin irritation to anaphylaxis, shock, and death. To avoid allergic responses, allergy sufferers must avoid foods containing allergenic ingredients. As a result, customers rely on food labels to inform them of the presence of allergenic substances. Food makers must create, implement, and maintain the required controls to guarantee that allergens that are intended to be present in a food are reported on the label and that unintended allergies are not present. Advisory words such as “may contain [allergen]” or “produced on equipment that also processes [allergen]” are not sufficient to prevent allergen contact. Allergen management will be achieved through the use of prerequisite programs as well as HACCP plan controls that ensure accurate product labelling. As a result, in Serbia, the National Allergen Strategy (NAS) must be developed in collaboration with key stakeholder organizations in order to improve the health and quality of life of Serbians with allergic diseases, and to ease the burden of allergic diseases on individuals, healthcare services and the community.

Alergeni u hrani – opasnost u sistemu bezbednosti hrane

Jovanka Popov Raljić, Milica Aleksić, Vesna Janković

Apstrakt: Alergeni u hrani javljaju se u poslednje dve decenije kao prikriveni oblik pretnje koja značajno ugrožava javno zdravlje, a njihovo obeležavanje na prehrambenim proizvodima, pićima i proizvodima koji nisu prethodno upakovani je jasno definisano zakonskim propisima. Alergija na hranu (FA) je hronično stanje opasno po život koje značajno narušava kvalitet života. FA predstavljaju značajan zdravstveni problem koji pogađa decu i odrasle i predstavlja značajan teret za zdravlje, zdravstveni sistem i ekonomije u razvoju. FA se definiše kao štetni zdravstveni efekat koji proizilazi iz specifičnog imunološki posredovanog odgovora koji se javlja prilikom konzumiranja određene namirnice, a koja može biti posredovana IgE antitelima specifičnim za hranu, ćelijski mehanizam ili oba. Odgovarajuće upravljanje alergijama na hranu postalo je problem za prehrambenu industriju zbog sve većeg broja osoba sa alergijama na hranu.

Ključne reči: *alergije na hranu, alergeni, velikih 8.*

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Original scientific paper

Evaluation of Chemical Analyses of Experimentally Prepared Fermented and Heat-Treated Sausages

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A b s t r a c t: Sausages are very open to adulteration because of their preparation procedures. The purpose of this study was to examine the chemical composition of fermented and heat-treated sausages prepared experimentally with the addition of tissues and organs that are likely to be used in adulterated meat products and to determine whether the chemical analyses provided adequate information about sausage composition. The experimental fermented or heat-treated sausages were prepared with the addition of tissues and organs (seven tissue/organ combinations were studied; head meat-lung, tongue-liver, trachea-rumen, spleen-intestine, mammary gland-brain, heart-testis and kidney-oesophagus) that are likely to be used to adulterate sausage meat products. Appropriate control sausages not containing any organ additions were prepared according to the Turkish Food Codex. The most remarkable result is that contents of moisture, fat, ash and total protein are not sufficient criteria to determine the quality of the sausages. However, hydroxyproline content is an important criterion for the detection of collagen tissue, and this chemical analysis must be supplemented by histological analysis in future studies.

Keywords: *chemical analysis, fermented sausages, heat-treated sausages, hydroxyproline.*

Introduction

Meat is a nutrient source that is extremely rich in protein, vitamins and minerals, but is low in carbohydrates. At the same time, the nutrients in meat exist in vegetables at low levels, or their bioavailability is extremely poor. Meat has important nutrients for adequate and balanced nutrition and for the protection of human health and prevention of diseases (*Muguerza et al.,* 2004; *Biesalski,* 2005; *Ekici and Ercoşkun,* 2007). Meat, with its high amounts of water and a favourable pH, is a suitable medium for the development of microorganisms (*Nychas and Arkoudelos,* 1990; *Serdaroğlu and Sapancı Özsümer,* 2000). Thus, various products have been manufactured in different societies throughout history to increase meat durability and to provide different flavours and aromas. The methods used in meat production are salting, drying, curing, cooking, cooling, freezing, fermentation, heat-treatment, irradiation and addition of chemicals to the meat (*Çon et al.,* 2002; *Öztan,* 2013; *Anar,* 2015). Fermentation is one of the oldest methods used for long-term preservation of foods (*Caplice and Fitzgerald,* 1999; *Nassu et al.,* 2003). When fermentation is applied together with the methods of adding salt and reducing water, high quality products are made, and effective protection is achieved as well (*Molly et al.,* 1997; *Çiçek et al.,* 2015).

Fermented sausage is one of the oldest meat product types manufactured in Turkey, and its processing technology resembles that of fermented dry salami and sausages produced in Europe and America (*Gökalp et al*., 2004). In addition to fermented sausages, heat-treated sausage production occurred in time, and recently, with developed technologies, these products have become common (*Güner et al.,* 2011; *Pehlivanoğlu et al.,* 2015). Sausages are meat products that are open to adulteration due to their production methods (*Güçer and Gövercin,* 2010; *İnceand Özfiliz*, 2018). To prevent adulteration and provide appropriate standards in meat production, in 1860, legislative regulations were developed and implemented for the first time in England, and other countries later followed these regulations. To determine the adulteration of meat products, physical, chemical, microbiological and histological standards have been reported (*Ekici and Ercoşkun,* 2007). In countries where sausage products are widely consumed, a number of studies have examined wheth-

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er products comply with these physical, chemical, microbiological standards (*Salgado et al.,* 2006; *Marcinčák et al.,* 2014).

The purposes of this study were to examine the chemical composition of fermented and heat-treated sausages prepared experimentally with the addition of tissues and organs that are likely to be used to produce adulterated sausages products and to determine whether the chemical analyses provide adequate information about sausage quality.

Materials and Methods

As controls, both fermented and heat-treated sausages were formulated with beef meat and fat without additives according to the Turkish Food Codex. In the experimental groups, sausages were formulated with the addition of two tissues and/or organs in equal proportions of 10% to make up a total of 20% (seven tissue/organ combinations were studied; head meat-lung, tongue-liver, trachea-rumen, spleen-intestine, mammary gland-brain, heart-testis and kidney-oesophagus). The fermented sausages were fermented for 8 days under air flow at 0.5–1 m/second, relative humidity of 75–90% and at 18–22°C until maturation. On the other hand, the heat-treated sausages were subjected to a heating process in a baking oven until a central temperature of 68°C was reached. Following these procedures, the sausages were vacuum-packed and stored at 4°C.

Chemical analyses

All chemical analyses were conducted acccording to standard methods (*AOAC*, 2003), as detailed below. Sausage material containing ca. 2 g dry matter was dried in mechanical convection oven at 105°C for 12 h. After drying, each sample was cooled in a desiccator and weighed. The loss in weight was recorded as the amount of moisture (AOAC 950.46). Approximately 2 g sausage sample was put into a porcelain crucible and burned in an ash oven at 550°C for 6 h. After burning, the sample was cooled in a desiccator and weighed. The weight lost was subtracted from the initial sample weight and the remaining weight was recorded as the amount of crude ash (AOAC 923.03). Fat content was measured by the Soxhlet system. Roughly 4 g of sausage sample was weighed onto filter paper and subjected to extraction in the Soxhlet extractor for 6 h. The solvent used was diethyl ether, and the extract was collected in a suitable container. When the extraction process ceased, the container was dried in an oven at 105°C for 1 hour and subsequently cooled in a desiccator. After cooling down, the remaining extract was recorded as the amount of fat (AOAC 960.39). Protein content was measured by the Kjeldahl (N×6.25) method. Approximately 1 g of sausage sample was digested at high temperature with 98% concentrated sulphuric acid to convert nitrogenous substances to ammonium salts. Then, ammonium salts were converted to free ammonia by applying 40% concentrated NaOH. Ammonia was titrated with hydrochloric acid to determine the N content. Since N forms 16% of protein molecule, the N content found was multiplied by 6.25 to calculate amount of total protein (AOAC 928.08). 4-Hydroxyproline was used as an indicator of collagen in the sausage samples. Therefore, 4 g of the homogenate obtained from a 200 g sausage sample was transferred to an Erlenmeyer flask and hydrolysed with sulphuric acid at 105°C for 16 h. After hydroxylation, the hydrolysate was diluted with distilled water and filtered. The concentration of 4-hydroxyproline was measured by spectrophotometer (AOAC 990.26).

Statistical analyses

Statistical analyses were conducted in the SPSS 23.0 package program (*International Business Machines*, 2015). The normal distribution of continuous variables was evaluated by Shapiro-Wilk test. A single sample *t*-test was used for comparisons of two groups of variables that were not normally distributed. The descriptive statistics of the continuous variables are shown by mean and standard deviation values and categorical variables by frequency and percentage. Comparisons below $p<0.05$ were considered statistically significant in all statistical analyses in the study.

Results and Discussion

Fermented and heat-treated sausages were evaluated in terms of moisture, ash, fat, total protein and hydroxyproline contents for the purpose of determining quality. The results are shown in Tables 1 and 2.

Moisture levels of control sausages and the mean of the test groups were 54.72% and 55.50%, respectively, in fermented sausages and 54.46% and 56.37%, respectively, in heat-treated sausages. Our results are higher than the following results; 43.08% moisture levels for fermented sausages in the Istanbul markets, 44.60% in Spanish Androlla sausage, 47.58% in Afyon province sausages, 20.78% in fer-

mented sausages sold in Kahramanmaraş province, 38.75% in the Elazığ markets, 32.20% and 29.76% in Spain in homemade and industrial sausages, respectively, and 42.33% in Bosnian sausages (*Pehlivanoğlu et al.,* 2015; *Lorenzo et al.,* 2000; *Doğu et al.,* 2002; *Erdoğrul and Ergün,* 2005; *Öksüztepe et al.,* 2011; *Salgado et al.,* 2006; *Operta and Smajic,* 2012). The average moisture content of Botillo, produced in Spain, was 55.90%, which is similar to our results (*Lorenzo et al.,* 2000).

The amount of ash was 1.81% and 1.45% in fermented control and experimental sausages (means are presented), respectively, and 1.84% and 1.72% in heat-treated control and experimental sausages, respectively. In our study, no cartilage or bone tissue-containing pieces of meat were added to the control or to the experimental sausages, which resulted in no difference between the amounts of ash in the control and experimental sausages. In 1982–2016 in Turkey, the amount of ash in fermented sausages ranged from 3.85% to 5.88% (*Ertaş*, 1982; *Ertaş and Kolsarıcı,* 1983; *Kolsarıcı et al.,* 1986; *Sancak et al.,* 1996; *Atasever et al.,* 1998; *Erdoğrul and Ergün,* 2005; *Öksüztepe et al.,* 2011). The lower ash contents obtained in our study may be due to the absence of cartilage and bone tissue, which are not allowed in sausages in Turkey, but which were identified in histological studies on Turkish sausages (*İnal*, 1992; *Atasever et al.,* 1999; *Erdoğrul*, 2002; *Erdost et al.,* 2016; *İnce and Özfiliz*, 2016). The amounts of ash were 5.63% and 5.32% (*Salgado* et *al.,* 2006) in Spanish homemade and industrial sau-

Table 1. Chemical composition of fermented sausages

Sausage type	Moisture %	Ash %	Fat %	Total Protein %	Hydroxyproline (mg/100g)
Cf	54.72	1.81	19.98	21.92	116
Ef1	55.41	1.43	16.93	23.24	389
Ef2	55.86	1.42	17.32	22.20	375
Ef3	54.93	1.51	18.10	21.30	379
Ef4	58.54	1.40	18.04	20.71	355
Ef5	57.68	1.46	19.33	21.82	367
Ef6	51.38	1.67	18.59	24.54	371
Ef7	54.72	1.29	18.50	23.30	358
MEf	55.50±2.31	1.45±0.12	18.12±0.81	22.44±1.32	370.57±11.86

Legend: Cf – Control Turkish type fermented sausage; Ef – Experimental Turkish type fermented sausages; MEf – Mean of experimental Turkish type fermented sausages

Table 2. Chemical composition of heat-treated sausages

Sausage type	Moisture %	Ash %	Fat %	Total Protein %	Hydroxyproline (mg/100g)
Ch	54.46	1.84	14.92	22.06	126
Eh1	56.90	1.56	13.88	22.27	399
Eh2	55.02	1.66	15.03	22.33	381
Eh3	55.70	1.45	15.97	21.53	402
Eh4	58.20	1.72	15.68	22.14	357
Eh5	58.03	1.83	12.63	21.69	358
Eh6	54.49	1.96	16.64	23.62	375
Eh7	56.26	1.85	11.77	23.46	384
MEh	56.37±1.43	1.72±0.18	14.51±1.81	22.43±0.81	379.43±17.79

Legend: Ch – Control Turkish type heat-treated sausage; Eh – Experimental Turkish type heat-treated sausage; MEh – Mean of experimental Turkish type heat-treated sausages

sages, respectively, and 4.95% in the study reported by Operta and Smajic (2012) in Bosnian sausages. This difference in ash content between our study and these other studies can be attributed to the use of MRM (mechanically recovered meat) and stripping meat, which are prohibited in Turkey, but are allowed in Europe (*Tremlová et al.,* 2006; *Komrska et al.,* 2011).

The amounts of fat in the control and test groups (means) were determined as 19.98% and 18.12%, respectively, in fermented sausages and 14.92% and 14.51% respectively, in heat-treated sausages. Studies in Turkey on the amount of fat in fermented sausages have found an average between 28.09% and 39.97% of fat (*Ertaş*, 1982; *Ertaş and Kolsarıcı*, 1983; *Kolsarıcı et al.,* 1986; *Sancak et al.,* 1996; *Atasever et al.,* 1998; *Çon and Gökalp,* 1998; *Doğu et al.,* 2002; *Erdoğrul and Ergün*, 2005; *Öksüztepe et al.,* 2011). In homemade and industrial sausages and in Androlla and Botillo in Spain, the average fat content was 46.50%, 47.85%, 21.22%, and 14.82%, respectively (*Lorenzo et al.,* 2000; *Salgado et al.,* 2006). In a study of Bosnian sausages, the average amount of fat was 25.28% (*Operta and Smajic,* 2012). Animal fats are the source of essential fatty acids and fat soluble vitamins (*Ekşi and Ertaş*, 2011).

In the control and experimental groups, the total amounts of protein were determined as 21.92% and 22.44%, respectively, in fermented sausages and 22.06% and 22.43%, respectively, in heat-treated sausages. In 1982–2015, for fermented sausages produced in Turkey, the protein levels were between 17.16% and 27.3%, whereas the results in our current study are at the lower end of this spectrum (*Ertaş*, 1982; *Ertaş and Kolsarıcı*, 1983; *Kolsarıcı et al.,* 1986; *Sancak et al.,* 1996; *Atasever et al.,* 1998; *Doğu et al.,* 2002; *Erdoğrul and Ergün*, 2005; *Öksüztepe et al.,* 2011; *Pehlivanoğlu et al.,* 2015). Our results for total protein were similar to Spanish Androlla at 22.66%, lower than Bosnian sausages at 26.57%, higher than Botillo at 17.73%, homemade sausages at 13.69%, and Spanish industrial sausages at 15.16% (*Lorenzo et al.,* 2000; *Salgado et al.,* 2006; *Operta and Smajic,* 2012).

In terms of total protein content (%), there was no statistically significant difference between experimental sausages and control group (means) in both fermented ($p = 0{,}335$) and heat-treated sausages ($p = 0.268$), as shown in Figure 1. These results show that the control and experimental groups prepared by adding tissue and organs contained similar amounts of protein.

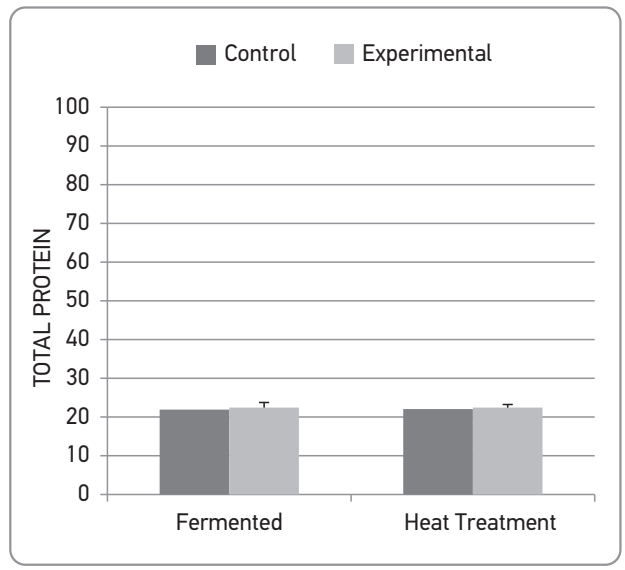

Figure 1. Comparison of total amounts of protein level (%).

The amounts of hydroxyproline in the control and experimental groups (means) were 116 mg/100 g and 355 to 389 mg/100 g, respectively, in fermented sausages, and 126 mg/100 g and 357 to 402 mg/100 g, respectively, in heat-treated sausages. In Turkey hydroxyproline levels in fermented sausages have reported in the ranged of 127mg-530mg/100g (*Ertaş and Kolsarıcı*, 1983; *Kolsarıcı and Ertaş*, 1986; *İnce et al.,* 2018), while in other countries, the levels ranged from 90mg/100g to 970mg/100g (*Lorenzo et al.,* 2000; *Salgado et al.,* 2006; *González-Martín et al.,* 2009; *Mazorra-Manzano et al.,* 2012).

In terms of the hydroxyproline levels, there was a statistically significant difference between

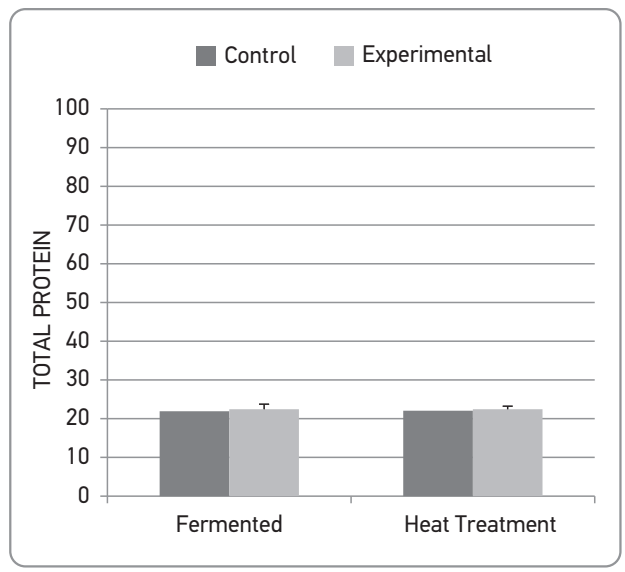

Figure 2. Comparison of hydroxyproline level (mg/100g).

the experimental and control groups (means) of fermented ($p < 0.001$) and heat-treated ($p < 0.001$) sausages, as shown in Figure 2. The results indicate that the addition of tissues and organs, except for muscle tissue, to sausages affects the level of hydroxyproline. We suggest that this result may be due to the amount of connective tissue-derived collagen.

However, our statistical results regarding total protein and hydroxyproline levels indicated that the total protein content of the fermented and heat-treated sausages is not sufficient to provide information on the quality and bioavailability of the protein (*Kolsarıcı and Ertaş,* 1986; *González-Martín et al.,* 2009). Studies conducted by *Brito et al.* (2010) on traditional and industrial sausages showed that the total protein ratios and hydroxyproline levels of traditional sausages were high, while in sausage samples examined by *Kurćubić et al.* (2012), the total protein ratio was low and the hydroxyproline level was high. Thus, the hydroxyproline level is important. However, the hydroxyproline results do not address the issue of whether the products sold on markets are poor quality meat or whether different tissues and organs were used to adulterate the sausages. Although the levels of hydroxyproline show the presence of connective tissue-derived collagen tissue, for indicating from which tissues or organs the collagen originates, histological examinations must be conducted (*Atasever et al.,* 1999; *Erdoğrul*, 2002; *Sezer et al.,* 2013; *Latorre et al.,* 2015; *İnce and Özfiliz,* 2016).

Conclusions

The results of our study show that the moisture, fat, ash and total protein analysis results do not differ between the control and organ-added experimental groups of sausages. However, there was a statistically significant difference in hydroxyproline levels between the control and organ-added sausages. The most remarkable result is that moisture, oil, ash, and total protein contents are not sufficient criteria to determine the quality of the sausage products.

It was also concluded that hydroxyproline analysis is an important criterion for the detection of collagen tissue from connective tissue proteins in sausages, while histological analysis must be conducted to determine the origin of the adulteration.

Ocena hemijskih analiza eksperimentalno pripremljenih fermentisanih i termički obrađenih kobasica

Eser İnce, Mukaddes Merve Efil, Nesrin Özfiliz

A p s t r a k t: Kobasice su veoma otvorene za patvorenje (oponašanje) zbog postupka njihove pripreme. Svrha ovog istraživanja je bila da se ispita hemijski sastav fermentisanih i termički obrađenih kobasica pripremljenih eksperimentalno uz dodatak tkiva i organa koji će se verovatno koristiti u patvorenim proizvodima od mesa i da se utvrdi da li su hemijske analize dale adekvatne informacije o sastavu kobasica. Eksperimentalne fermentisane ili termički obrađene kobasice pripremane su uz dodatak tkiva i organa (proučavano je sedam kombinacija tkivo/organ; meso glave -pluća, jezik-jetra, dušnik-burag, slezina-crevo, mlečna žlezda-mozak, srce- testis i bubreg-jednjak) koji će se verovatno koristiti za patvorenje mesnih proizvoda od kobasica. Odgovarajuće kontrolne kobasice bez dodataka organa pripremljene su prema turskom kodeksu za hranu. Najznačajniji rezultat je da sadržaj vlage, masti, pepela i ukupnih proteina nije dovoljan kriterijum za određivanje kvaliteta kobasica. Međutim, sadržaj hidroksiprolina je važan kriterijum za detekciju kolagenog tkiva, a ova hemijska analiza mora biti dopunjena histološkom analizom u budućim istraživanjima.

Ključne reči: *hemijska analiza, fermentisane kobasice, termički obrađene kobasice, hidroksiprolin*

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Modelling and optimization of the quality indices for the production of ingredient-mix based dried chicken product (chicken *kilishi*)

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A b s t r a c t: This study was designed to investigate, model and optimise the influence of cooking treatments (untreated, boiling and steaming), ingredient-mix infusion temperature (30°C, 40°C and 50°C) and infusion duration (5, 10 and 15 min) on the quality indices of *ingredient-mix based dried chicken product (chicken kilishi)* produced from chicken breast. *The quality indices (proximate composition and mineral contents) of the produced* chicken kilishi *were determined and analysed statistically using a hybrid Taguchi-Response Surface Methodology design. The production of* chicken kilishi *from untreated chicken breasts favours an increase in ash content (9.04%), crude protein content (50.28%) and zinc content (16.34 ppm). However, the* chicken kilishi *produced from the steaming treatment gives the highest fat content (14.02%), carbohydrate content (28.97%) and phosphorus content (12.07 ppm), while the kilishi subjected to the boiling treatment have increased iron content (18.24 ppm) but decreased moisture content (5.32%). The developed polynomial regression models for the quality indices were significant with* R^2 *and* R^2_{adj} *that ranges from 0.88 to 1.00, respectively. The optimum process conditions were attained when the chicken breasts were not treated and* ingredient-mix *infusion was conducted for 6 min at 41°C.*

Keywords: *chicken breast; chicken kilishi; modelling; optimization; quality indices.*

Introduction

Ingredient-mix based dried meat is also known as *kilishi*. This is one of the most popular dried meat snacks produced in developing countries. It is a traditional sun-dried Nigerian and Sahara African meat product obtained from defatted lean beef meat in conjunction with spices of plant origin (*Abubakar et al.,* 2011). It constitutes one of the daily delicacies in the region and is equivalent to pemmican and charqui consumed in South and Western America. *Kilishi* contains about 46% meat and 54% non-meat ingredients (*Iheagwara and Okonkwo,* 2016).

Chicken and other poultry products are consumed for their nutritional qualities and characteristics flavour in all countries of the world (Latshaw and Musharaf, 2007). Chicken is the most common meat type in the poultry category. Nevertheless, turkey and duck also are important poultry meats (FAO, 2017). In most developed countries of the world, chicken meat is usually cut into different types and forms ranging from the complete and whole carcass, cut-up parts like breast, drumstick, wings, whole legs, thighs and other cuts or is further processed into chicken meat products.

Chicken meat provides a high amount of minerals; selenium, phosphorus, zinc, iron, potassium, magnesium and vitamins of B complex as well as A, C and K (Fernandes and Rodrigues, 2008). Meat produced in the northern part of Nigeria is processed into numerous products, including snack products such as kilishi, tsire, dambunnama and balangu, with differing physiochemical, sensory attributes and shelf life (Muhammad et al., 2011). Seini et al. (2018) produced kilishi from dried meat and evaluated its effect on the nutritional and microbiological characteristics of the product. Seydou et al. (2019), also compare the physicochemical properties of two types of beef manufactured kilishi and a sliced grilled meat. Iheagwara et al. (2021) evaluated the textural, rheological and sensory properties of ingredient-mix dried beef (kilishi). However, literature is sparse on the use of chicken breast to produce ingredient-mix dried chicken snacks, also known as chicken kilishi.

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Chicken is an excellent source of protein providing about 10% of total daily needs (*Soares et al.,* 2017). A need to improve the nutrition attributes of chicken meat calls for producing value-added products with improved nutrition composition compared to that of chicken alone that would meet consumer needs. Food additives and ingredients rich in nutrients such as ginger, clove and condiments can be added and processing techniques used which could improve the nutritional composition. The development of an ingredient-mix dried chicken snack (*kilishi)* with high nutritional value would greatly limit consumer dependence on beef. The in-depth understanding of the optimum processing technique for producing a chicken *kilishi* would aid in the development of a functional product with a high nutritional composition that can improve the health of the consumers.

Modelling is a technique that is used in food engineering to predict the future of food processes and products with good accuracy depending on the purpose of the model (*Trystram*, 2012). *Perrot et al*. (2011) reported that food process modelling is an important technique to understand, design and control food processes. The use of Taguchi and Response Surface Methodology has been widely reported as a statistical techniques meant for experimental designs, developing models, evaluating the effects of variables on response and searching for the optimum conditions (*Hussein et al.,* 2019; *Sanusi and Akinoso*, 2021). *Sanusi et al.* (2020) reported that one of the advantages of Taguchi is that it could be used to minimise the number of experimental runs while Response Surface Methodology could be used to establish the linear, quadratic and interaction effects of independent variables on responses or dependent variables (*Sanusi and Akinoso*, 2020). The modelling and optimisation of the cooking treatment, ingredient-mix infusion temperature and duration (hereafter called time) using a hybrid of Taguchi-Response Surface Methodology approach would guide producers on how to predict the quality indices of the chicken *kilishi* snack and also help in establishing its optimum condition. Thus, considerable savings can be achieved in processing time, cost and establishing a laboratory system for monitoring product quality. Therefore, the objective of this study was to investigate, model and optimise the influence of cooking treatment, infusion temperature and infusion time on the quality indices of chicken *kilishi* using a hybrid Taguchi-Response Surface methodology technique.

Materials and Methods

Ingredient-mix preparation

The recipe used for the ingredient-mix formulation is presented in Table 1. Red pepper, chilli pepper, sweet pepper, whole ginger, garlic and onion were first blended using a blender (Model: SFP 2203, Japan) to form a liquor. Thereafter, granulated black pepper, clove, seasoning, locust bean con-

Table 1. Recipe for ingredient-mix preparation

Ingredient/spice common name	Scientific name	Mass (kg)
Groundnut paste	*Arachis hypogea*	28.50
Black pepper	*Piper guineense*	3.40
Red pepper	*Capsicum frutescens*	2.30
Sweet pepper	*Capsicum annum*	1.90
Chilli pepper	*Afromomum meleginata*	2.10
Cloves	*Eugenice caryophylceta*	2.60
Whole ginger	*Zingiber officinale*	3.80
Garlic	*Allium sativum*	0.10
Palm oil	*Elaeis guineensis*	5.00
Vegetable oil	*Arachis hypogaea*	5.00
Onion	*Allium cepa*	8.40
Locust bean condiment (Iru)	*Parkia biglobosa*	0.40
Salt	*Sodium chloride*	0.70
Seasoning	*Monosodium glutamate*	5.80
Water		30.00

diment (*Iru*), salt, palm oil and vegetable oil were added and further blended. To form a suitable ingredient-mix texture, a controlled amount of groundnut paste and water was finally added and blended.

Chicken breast preparation

Fresh *ZARTECH* chicken breasts of 13.5 kg were purchased from Shoprite mall, Ilorin, Nigeria. The chicken breasts were carefully cut and trimmed into a thin, flat sheet of 2 mm thickness. The weight of each flat chicken sheet was recorded.

Experimental design

A hybrid of Taguchi experimental design and response surface methodology was used to study the influence of processing technique on the quality indices of chicken kilishi snack using Minitab version 16, U.K. Table 2 shows the experimental outline for the interactions of the cooking methods, ingredient-mix infusion temperature and infusion time on chicken kilishi using Taguchi design, while Response Surface Methodology (RSM) was used to evaluate the effect of the cooking method, infusion temperature and infusion time on the quality indices of chicken kilishi as shown in Table 2. The quality indices were related to the cooking method, infusion temperature and time by the second-order polynomial model of RSM that has linear, quadratic and interaction relationships as shown in Equation 1.

$$Y = C + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1 x_1^2 + \\ + \beta_2 x_2^2 + \beta_3 x_3^2 + \beta_{12} x_{12} + \beta_{13} x_{13} \quad (1)$$

where C is the coefficient of the model constant, β_1, β_2 and β_3 are the linear terms, $\beta_1 x_1$, $\beta_2 x_2$ and $\beta_3 x_3$ are the quadratic terms, $\beta_{12} x_{12}$, $\beta_{13} x_{13}$ are the interaction terms, Y is the response for each model (proximate composition, phosphorous, zinc and iron), and x_1, x_2 and x_3 are cooking method, infusion temperature and infusion time, respectively. Analysis of variance (ANOVA) was used to find the interactions between the processing conditions and responses (proximate composition and phosphorous, zinc and iron content), and the p-values at 95% confidence level and Fischer-values (F-value) were determined. The fitness of the models for the responses was determined by the coefficient of determination, R^2 and R^2_{adj}.

Production of chicken kilishi from chicken breast

The flow chart for the production of chicken *kilishi* is presented in Figure 1. The thin, flat 2 mm thick chicken breasts were weighed into 500 g amounts in three separate lots. The first lot was spread on red trays without cooking treatment. The second lot was boiled for 5 min in a 100°C water bath (Model 10–101, Dae Han Co, Korea), while the third lot was steamed for 10 min in a preheated convective oven at 120°C. The three lots (untreated, boiled and steamed chicken breast meat) were dried in a cabinet oven (Model AMP-9P, China) at 60ºC for 10 h. The drying was accomplished by regular turning over of the chicken breast sheet every 1 h to support even drying. The chicken breast sheets were removed from the oven and weighed. The dried pieces of chicken meat were then steeped in the prepared ingredient-mix for 5 min, 10 min and 15 min at an ingredient-mix temperature of 30ºC, 40ºC and 50ºC using the design in Table 1. The steeped chicken breasts were further dried in the cabinet oven (Model AMP-9P, China) for 5 h to heat-seal the ingredients on the products (*Kilishi*). The chicken kilishi samples were allowed to cool naturally on the

Table 2. Experimental design using Taguchi design

Cooking treatment	Infusion time (min)	Infusion temperature (°C)
Untreated	5	30
Untreated	10	40
Untreated	15	50
Boiling	5	40
Boiling	10	50
Boiling	15	30
Steaming	5	50
Steaming	10	30
Steaming	15	40

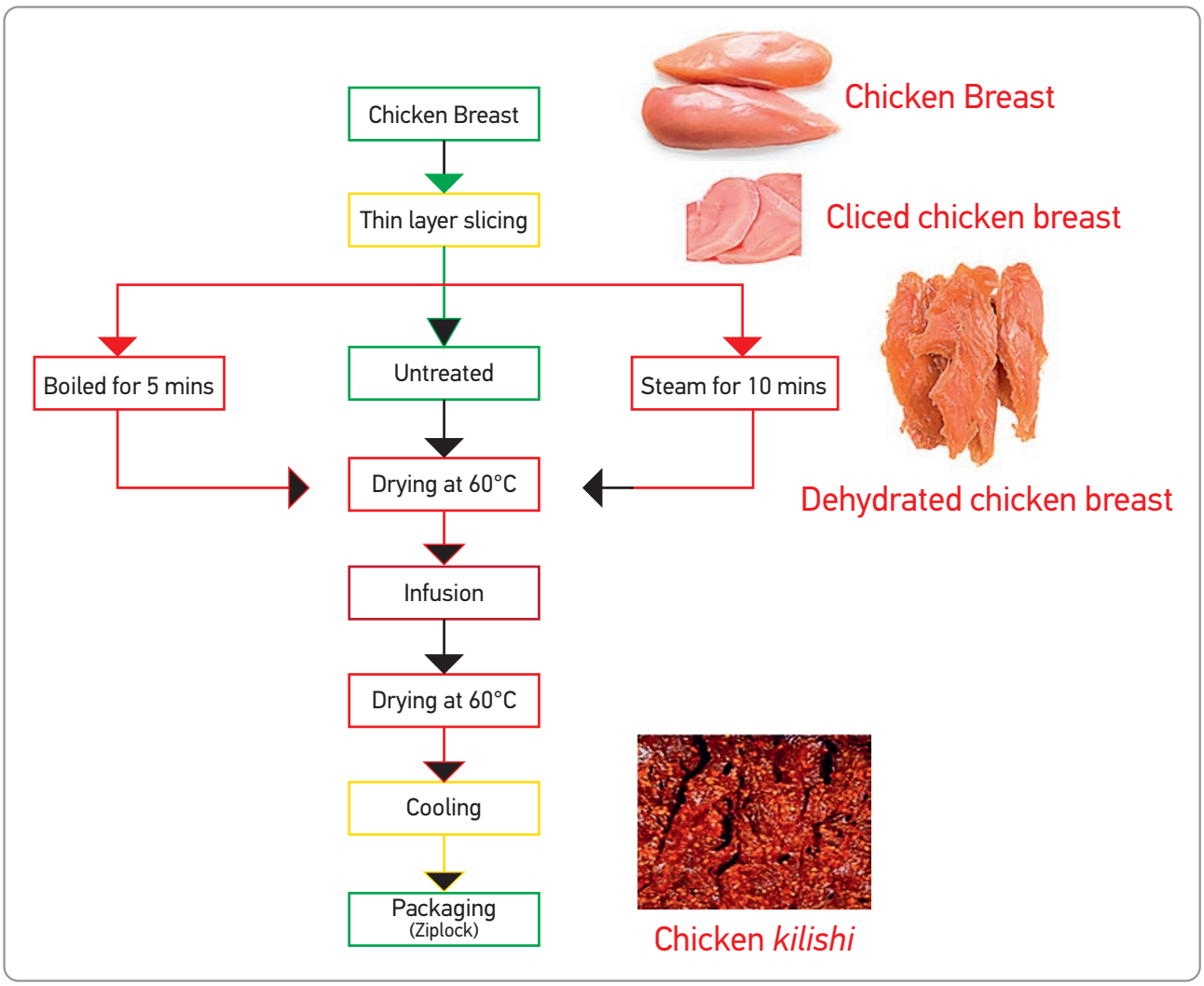

Figure 1. Flow chart for chicken *kilishi* production

trays, then were packed in Ziploc storage bags and stored until they were needed for analysis and sensory evaluation.

Quality indices

The proximate composition of the chicken *kilishi* samples was analysed using the *AOAC*, (2000) methods. For moisture content, method 925.09 was used, crude fat (Method 969.24), protein (Method 950.48, Nx6.25), and ash content (Method 923.03). The crude fibre was estimated according to *ISO* 5498:1981. The chicken *kilishi* was crumbled, mixed uniformly and a portion of the mixed material was taken to represent the whole *kilishi*. The analysis was carried out in triplicate. Phosphorous (P), zinc (Zn) and iron (Fe) contents in the samples were determined by Flame Atomic Absorption Spectrophotometer (VARIAN model AA240FS, United States) (*AOAC,* 2010).

Optimisation of the processing conditions

The second-order polynomial regression model was used to develop predictive model equations for the proximate composition and mineral content of the chicken *kilishi*. The cooking method, infusion temperature and infusion time were optimised using the desirability approach to maximise the crude protein content and minimise the moisture content. The Derringer and Suich methodology was used for the optimisation of the protein content and moisture content and was transformed into a desirability function. For perfect optimisation, the desirability values of the moisture content and crude protein content must be close to one (1). The second-order predictive models for moisture content and protein content were obtained, and the desirability function was calculated based upon the characteristics of using the following Equations 2 and 3 (*Sanusi and Akinoso*, 2020). Equation 2 and 3 were used for maximising the protein content and for minimising the moisture content, respectively.

$$d(y_i) = \begin{pmatrix} 0 & if\ y_i \le A_i \\ \left(\frac{y_i - A_i}{C_i - A_I}\right)^s & if\ A_i \le y_i \le C_i \\ 1 & if\ y_i \ge C_1 \end{pmatrix} \quad (2)$$

$$d(y_i) = \begin{pmatrix} 0 & if\ y_i \le A_i \\ \left(\frac{y_i - A_i}{C_i - A_I}\right)^t & if\ A_i \le y_i \le C_i \\ 1 & if\ y_i \ge C_1 \end{pmatrix} \quad (3)$$

The lowest acceptable and highest permissible values were represented by A_i, and C_i for moisture and crude protein content, respectively. The weights assigned to the moisture content and protein content was represented by s and t and was chosen to equal one. The individual desirability of moisture and protein content optimisation is d, while the targeted moisture and protein content is y_i. The composite desirability (Z) was used to jointly optimise the discrete desirability of moisture and protein content by finding the geometric mean using Equation 4 as described by *Sanusi and Akinoso* (2020).

$$Z = (d_1^{u_1} \times d_2^{u_2} \times d_3^{u_3} \times d_4^{u_4} \times d_5^{u_5} \times \ldots\ldots d_1^{u_1})^{\frac{1}{\sum u_i}} \quad (4)$$

where u_1 is the factor for moisture and crude protein content. Minitab 16 Statistical Software was used to compute the optimal solution that can guarantee desirable crude protein content and minimum moisture content.

Validation of the Optimum Processing Conditions

To validate the optimum processing conditions obtained from the response optimiser, the conditions were experimented with within the laboratory to determine their effects on crude protein content and moisture content of chicken *kilishi*. The experimental values from the laboratory and predicted values from the response optimiser were compared. The percentage errors were then determined by the validity of the optimisation as shown in Equation 5 as described by *Sanusi and Akinoso* (2021).

$$Percentage\ Deviation = \\ = \frac{(Experimental\ value - Predicted\ value)}{Predicted\ value} \times 100 \quad (5)$$

Sensory evaluation

Sensory evaluation of the chicken *kilishi* was carried out by 15 semi-trained panellists from the Department of Food Engineering, University of Ilorin, Nigeria. A nine-point hedonic scale was used with 9 representing 'extremely like' and 1 'extremely dislike'. The panellists were presented with the coded samples and were asked to judge the samples based on appearance, taste, flavour, spiciness, texture and overall acceptability.

Statistical analysis

Data were analysed using analysis of variance (SPSS-20). Means were separated using Duncan multiple tests. Significance was accepted at $p \le 0.05$. Replicated measurements were taken and values were recorded as means ± standard deviation (SD).

Results and Discussion

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – moisture content

The effect of cooking treatment, infusion temperature and infusion time on the proximate composition of chicken *kilishi* is shown in Table 3. The moisture content ranged from 5.32 to 11.75%. The least moisture content was observed in sample D (5.32%), wherein the sliced chicken breasts were treated by boiling before drying and infused in ingredient-mix for 5 min at 40°C, while sample G with the highest moisture content was obtained when the sliced chicken breasts were treated with steaming before drying, then infused in ingredient-mix for 5 min at 50°C. Figure 2 shows the contour plot for the effect of cooking treatment, infusion temperature and time on the moisture content of chicken *kilishi.* The highest moisture content, observed in sample G, could be attributed to the high infusion temperature (50°C) and the steaming treatment. The infusion of food material at a high temperature usually increases the rate of moisture absorption. The high amount of moisture content in the steaming treatment could be attributed to the formation of coating at the surface of the sliced chicken breasts which then prevents water loss during the steaming processing.

This result corroborates the findings of *Choi et al.* (2016), wherein the moisture content of marinated chicken steak that was treated with superheated steaming was higher than that treated by boiling. In the current study, for the sliced chicken breasts that were not treated before drying, it was observed that greater infusion time and temperature produced chicken *kilishi* with higher moisture content, while among the chicken *kilishi* that were produced from sliced chicken breasts that were treated by boiling before drying, greater infusion time resulted in high-

Table 3. Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken *kilishi*

Sample	Cooking treatment	Infusion time (min)	Infusion temperature (°C)	Moisture content (%)	Ash content (%)	Crude fat (%)	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)
A	Untreated	5	30	7.13±0.03^{f}	8.40±0.08^{b}	10.36±0.02^{h}	48.25±0.01^{c}	2.05±0.01^{bc}	23.81±0.05^{d}
B	Untreated	10	40	9.00±0.06^{d}	9.04±0.01^{a}	12.46±0.01^{b}	50.28±0.01^{a}	1.46±0.02^{e}	17.76±0.05^{g}
C	Untreated	15	50	10.74±0.01^{c}	8.35±0.02^{b}	12.35±0.01^{c}	50.08±0.05^{b}	1.63±0.00^{de}	16.86±0.06^{h}
D	Boiling	5	40	5.32±0.03^{i}	7.35±0.02^{d}	11.64±0.02^{f}	46.71±0.01^{e}	2.80±0.00^{a}	26.19±0.06^{c}
E	Boiling	10	50	6.11±0.02^{h}	8.16±0.04^{c}	10.57±0.02^{g}	46.21±0.00^{g}	2.31±0.01^{b}	26.64±0.03^{b}
F	Boiling	15	30	8.31±0.01^{e}	6.41±0.00^{g}	12.03±0.01^{e}	47.05±0.02^{d}	2.36±0.44^{b}	23.86±0.47^{d}
G	Steaming	5	50	11.76±0.08^{a}	6.31±0.02^{h}	12.31±0.01^{c}	46.24±0.00^{g}	1.54±0.01^{e}	21.84±0.09^{e}
H	Steaming	10	30	10.97±0.02^{b}	7.08±0.05^{f}	14.02±0.01^{a}	46.49±0.01^{f}	2.13±0.01^{bc}	19.30± 0.04^{f}
I	Steaming	15	40	6.45±0.03^{g}	7.226±0.01^{e}	12.19±0.04^{d}	43.23±0.01^{h}	1.94±0.09^{cd}	28.97±0.14^{a}

Legend: *Values with the same subscript letters in the same column have no significant difference ($p \geq 0.05$)

er moisture content of the final product. However, chicken *kilishi* produced from the sliced chicken breasts that were treated with steaming before drying had the highest moisture content, which was observed at a high infusion temperature (50°C) but at low infusion time. Hence, the variation in the infusion temperature, infusion time and cooking treatment accounted for the significant differences in the moisture content of the products. Low moisture content in food products would aid proper food preservation. The moisture contents observed in the chicken *kilishi* were either lower or within the range of 10.00 to 12.02% that was reported by *Ogunsola and Omojola* (2008) and *Iheagwara and Okonkwo* (2016) for beef *kilishi*. Therefore, sample D with the least moisture content is expected to have a longer shelf life and be less prone to microbial spoilage than sample G.

The second-order polynomial regression equation for the effect of cooking treatment, infusion temperature and infusion time on the moisture content of chicken *kilishi* is presented in Equation 7. The predictive model equation for the moisture content showed that cooking treatment (X_1), infusion temperature (X_2), double effect of infusion time and interaction of cooking treatment and infusion time signifies that decrease of these parameters will increase the moisture content of chicken *kilishi*.

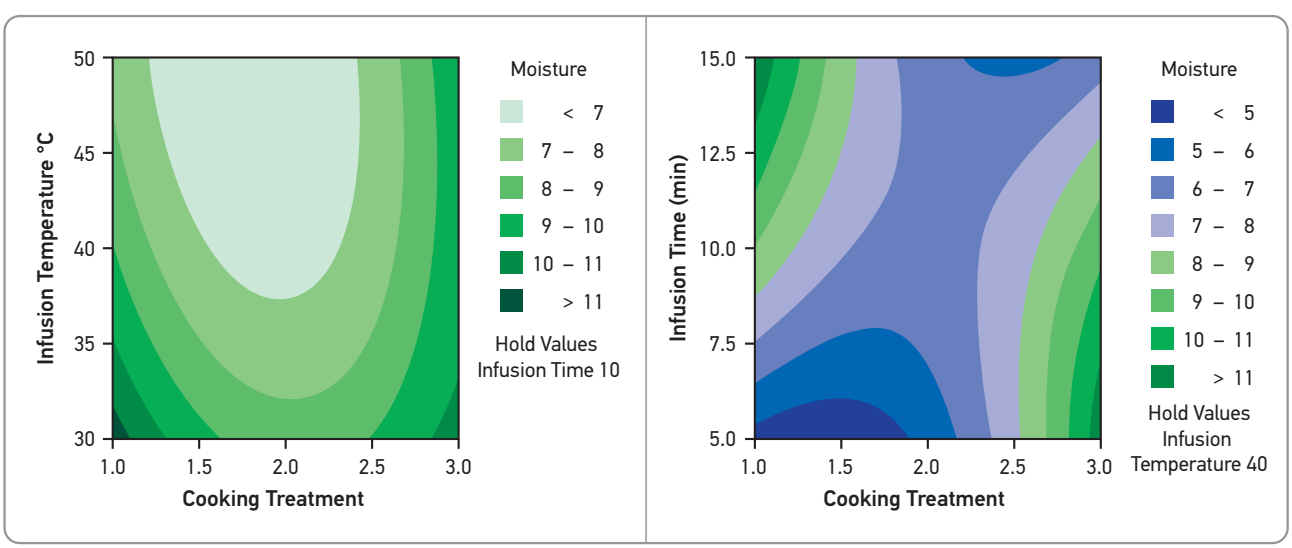

Figure 2. Effect of cooking treatment, infusion temperature and time on the moisture content of chicken *kilishi*

Moreover, increase in infusion time (X_3), the double interaction effect of cooking treatment, double interaction effect of infusion temperature and the interaction of cooking treatment and infusion temperature would increase the moisture content of the product. The R^2 and $R^2_{(adj)}$ obtained from the polynomial regression model in Equation 7 were 0.99 and 0.99 while the p-value and F-value were 0.000 and 7121.25, respectively. This shows that the polynomial regression equation is capable of predicting the effect of cooking treatment, infusion temperature and infusion time on the moisture content of chicken *kilishi*.

$$Moisture\ content\ = 21.439 - 7.138X_1 - \\ - 0.801X_2 + 1.960X_3 + 2.764X_1^2 + 0.007X_2^2 - \\ - 0.030X_3^2 + 0.068X_1X_2 - 0.625X_1X_3 \quad (6)$$

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – ash content

From Table 3, the ash content ranged from 6.31 to 9.04%. Sample B had the highest ash content (9.04±0.01%), wherein the sliced chicken breasts were untreated before drying, infused in ingredient-mix for 10 min at 40°C infusion temperature. The ash contents of samples A and C were not significantly different at p≥0.05, and with high ash contents, they ranked next to sample B. Therefore, the untreated *kilishi* had higher ash contents than the *kilishi* that were boiled or steamed. Figure 3 shows the effect of cooking treatment, infusion temperature and infusion time on the ash content of chicken *kilishi.* The ash contents that were obtained for the chicken *kilishi* samples in our current study were higher than the ash content of raw chicken breast (<2%) that was reported by *Chen et al.* (2016). This implies that cooking treatment and infusion with the ingredient-mix at different temperatures and for different times could improve the ash content of raw chicken breast. The ash content of the seasoned dried chicken snack samples obtained in this study was higher than 3.93 to 4.48%, which was reported for beef *kilishi* by *Inusa and Said* (2017) and 7.40 to 7.60% that was reported by *Daminabo et al.* (2013). The higher ash content could be attributed to the use of non-meat ingredients and the processing technique.

$$Ash\ content = 5.922 - 2.575X_1 + 0.183X_2 + \\ + 0.161X_3 + 0.429X_1^2 - 0.001X_2^2 - 0.023X_3^2 + \\ + 0.3388X_1X_2 + 0.135X_1X_3 \quad (7)$$

Equation 8 shows that a decrease (from steaming to not cooked) in cooking treatment severity increased the ash content. This could account for the chicken *kilishi* produced from untreated sliced chicken breasts having higher ash content than the steamed and boiled *kilishi*. Also, a decrease in the double interaction of infusion temperature and time increased the ash content. The increase in infusion temperature, infusion time, double interaction of cooking treatment, the interaction of cooking treatment and infusion temperature and interaction of cooking treatment and infusion time increased the ash content. The R^2 and $R^2_{(adj)}$ for the ash content of the polynomial regression model were 0.99 and 0.99 while the p-value and F-value were 0.000 and 1343.23, respectively. This shows that the polynomial regression model is capable of predicting the effect of cooking treatment, infusion temperature and infusion time on the ash content of chicken *kilishi*.

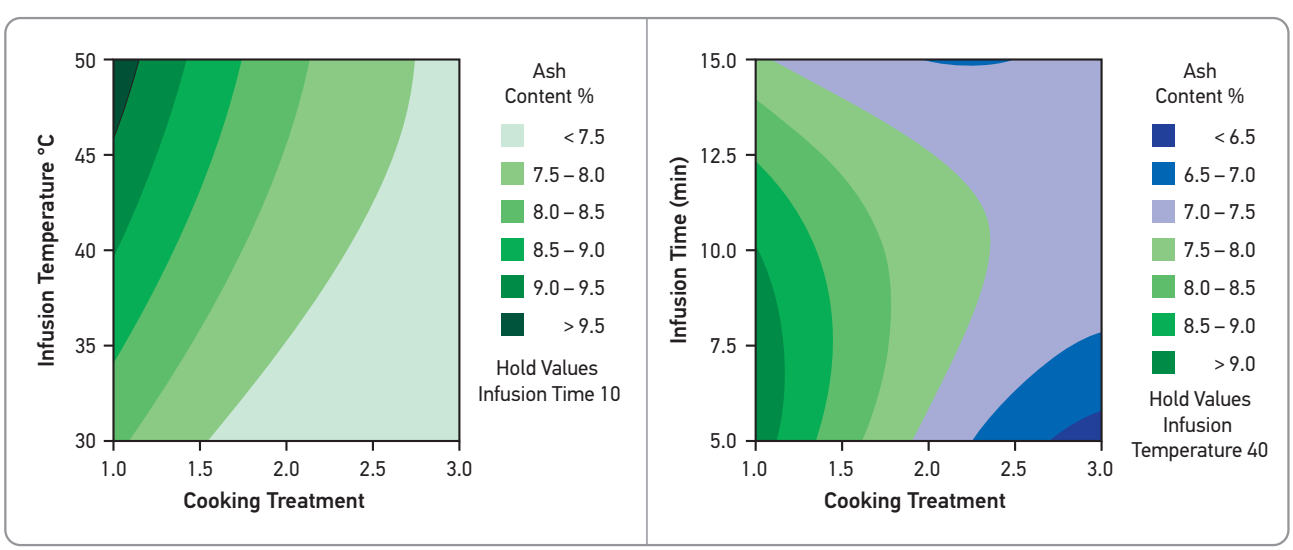

Figure 3. Effect of cooking treatment, infusion temperature and time on the ash content of chicken *kilishi*

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – crude fat content

The crude fat content (Table 3) of the chicken *kilishi* ranged from 10.36 to 14.02%. Sample H had the highest fat content (14.02%), and was produced by steaming the sliced chicken breasts before drying, ingredient-mix infusion at 30°C and 10 min infusion time. Figure 4 shows the effect of cooking treatment, infusion temperature and infusion time on the crude fat content of chicken *kilishi.* The crude fat content obtained in this study was higher than the 2.12 to 2.14% that was reported for chicken steaks under different cooking methods by *Choi et al.* (2016) and 1.12 to 2.03% that was reported for raw chicken breast by *Chen et al.* (2016). The higher crude fat content in sample H could be due to the low infusion temperature of the *Kilishi* at 30°C and infusion time of 10 min. This is in agreement with *Asmaa et al.* (2015), who reported an increase in fat content at a low cooking temperature and time. The application of high temperature makes the fat globules break up, form bonds with available radicals, and thereby, results in lower fat content. Sample A with the least crude fat content could be attributed to the fat solidification of the ingredient-mix at the very low infusion temperature and time. Therefore, the infusion temperature and infusion time needed for the fat in the ingredient-mix to be dissolved and absorbed by the sample was likely not reached. Thus, the fat absorption rate was probably slower than in the other samples. The crude fat content obtained in this study was higher than 8 to 10% reported by *Inusa and Said* (2017), but lower than 17.34 to 19.20% that was reported *Mgbemere et al.* (2011) for beef *kilishi.*

The polynomial regression model in Equation 9 shows that an increase in cooking treatment, infusion temperature, infusion time, double interaction of cooking treatment and interaction of infusion time and cooking treatment produced an increase in the crude fat content. This corroborates the findings of *Zzaman et al.* (2015), that fat is release as temperature and holding time increase. In the current study, however, decrease in the double interaction of infusion temperature and infusion time, and the interaction of cooking treatment and infusion temperature increased the crude fat content. The R^2 and $R^2_{(adj)}$ for the crude fat content of the polynomial regression model were 0.99 and 0.99 while the p-value and F-value were 0.000 and 7692.83, respectively. This shows that the polynomial regression model is capable of predicting the effect of cooking treatment, infusion temperature and infusion time on the crude fat content of chicken *kilishi* snacks.

$$Crude\ fat\ content = 4.399 + 1.1432X_1 + \\ + 0.612X_2 + 0.796X_3 + 0.870X_1^2 - 0.008X_2^2 - \\ - 0.014X_3^2 - 0.040X_1X_2 + 0.245X_1X_3 \quad (8)$$

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – crude protein

The crude protein levels obtained in the chicken *kilishi* ranged from 43.23 to 50.28% as shown in Table 3. The highest protein contents were found in samples A, B and C, which were not heat-treated by boiling or steaming. Figure 5 shows the effect

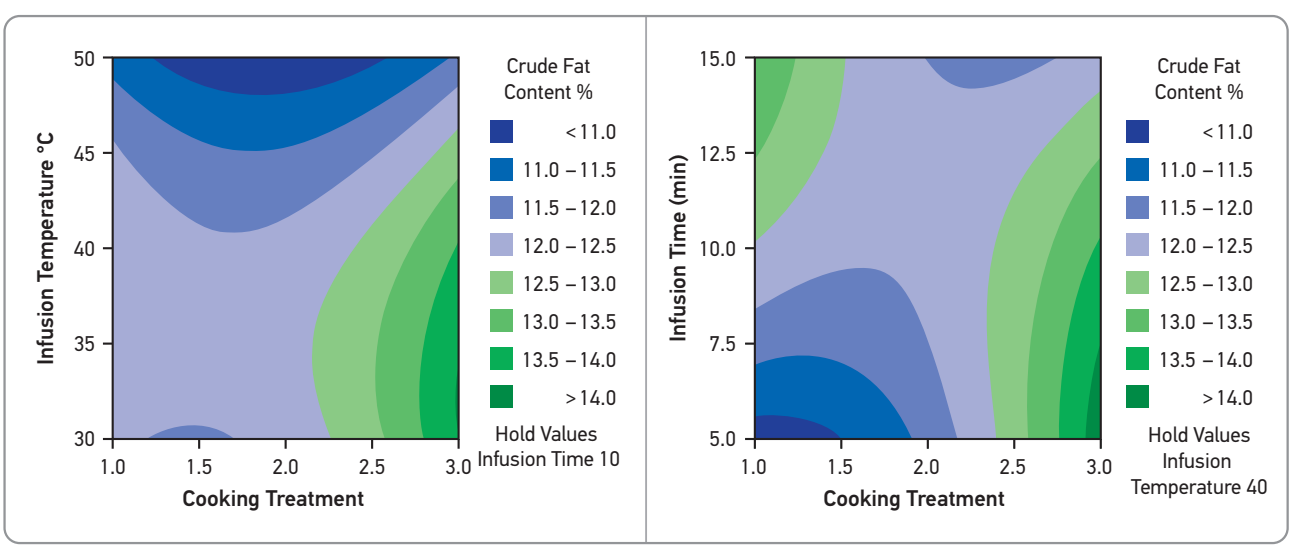

Figure 4. Effect of cooking treatment, infusion temperature and time on the crude fat content of chicken *kilishi*

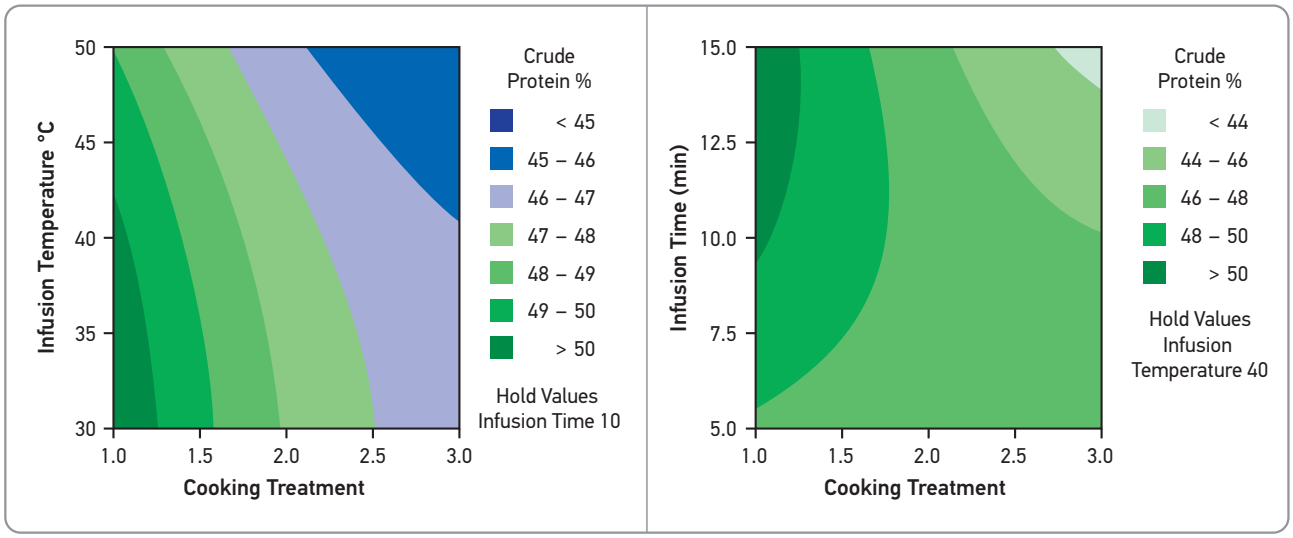

Figure 5. Effect of cooking treatment, infusion temperature and time on the crude protein content of chicken *kilishi*

of cooking treatment, infusion temperature and infusion time on the crude protein content of chicken *kilishi.* There was a clear significant difference in the protein content of the samples at $p<0.05$. The highest protein content was observed in sample B, wherein the sliced chicken breasts were not treated before preparation and had a 10 min infusion time at 40°C, while the least protein was measured in sample I, wherein the chicken breasts were treated by steaming for 15 min infusion time and at 40°C. This result agrees with *Seo et al.* (2016), who stated that superheated steam treatment contributes to the decrease in protein content of meat products. Also, *Choi et al.* (2016) reported that the protein content of chicken steak that was treated with boiling was higher in protein content than steamed treatment.

Bogosavljevic-Boskovic et al. (2010) and *Chen et al.* (2016) reported an average of 23% protein in raw chicken, which is much lower than the least level of protein that we measured in sample I (43.23%) of this study. The high protein content in the *kilishi* could be traced to the non-meat ingredients used in the ingredient-mix formulation. This is because the non-meat ingredients contain some ingredients that are naturally high in protein. *Ogunsola and Omojola* (2008), *Emmanuel et al.* (2020) and *Inusa and Said* (2017) reported 59–60%, 64% and 58.33–64.10%, respectively, for protein content in beef *kilishi*. These values were higher than the protein contents obtained in our current study. According to *Ogunsola and Omojola* (2008), raw beef contains 45% of protein while *Bogosavljevic-Boskovic et al.* (2010) reported an average of 23% of protein for raw chicken. Therefore, the initial protein content of beef meat might be responsible for the higher protein content obtained in beef *kilishi* than in the chicken *kilishi.*

The polynomial regression model in Equation 10 shows that an increase in infusion temperature, infusion time, double interaction of cooking treatment and interaction of infusion temperature and cooking treatment increased the crude protein content, while a decrease to less severe cooking treatment, the double interaction effect of infusion temperature and time, and interaction of cooking treatment and infusion time increased the crude protein content. The R^2 and $R^2_{(adj)}$ for the crude protein content of the polynomial regression model were 1.00 and 0.99 while the p-value and F-value were 0.000 and 24130.98, respectively. This shows that the polynomial regression model is adequate to effectively predict the effect of cooking treatment, infusion temperature and infusion time on the crude protein content of seasoned chicken dried meat.

$$Crude\ protein = 42.789 - 1.666X_1 + 0.162X_2 + \\ + 1.319X_3 + 0.773X_1^2 - 0.003X_2^2 - 0.031X_3^2 + \\ + 0.012X_1X_2 - 0.392X_1X_3 \quad (9)$$

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – crude fibre

The effect of cooking treatment, infusion temperature and infusion time on the crude fibre of chicken *kilishi* is presented in Table 3. Sample D had the highest (2.80%) and Sample B the lowest (1.46%) amount of crude fibre. Figure 6 shows the

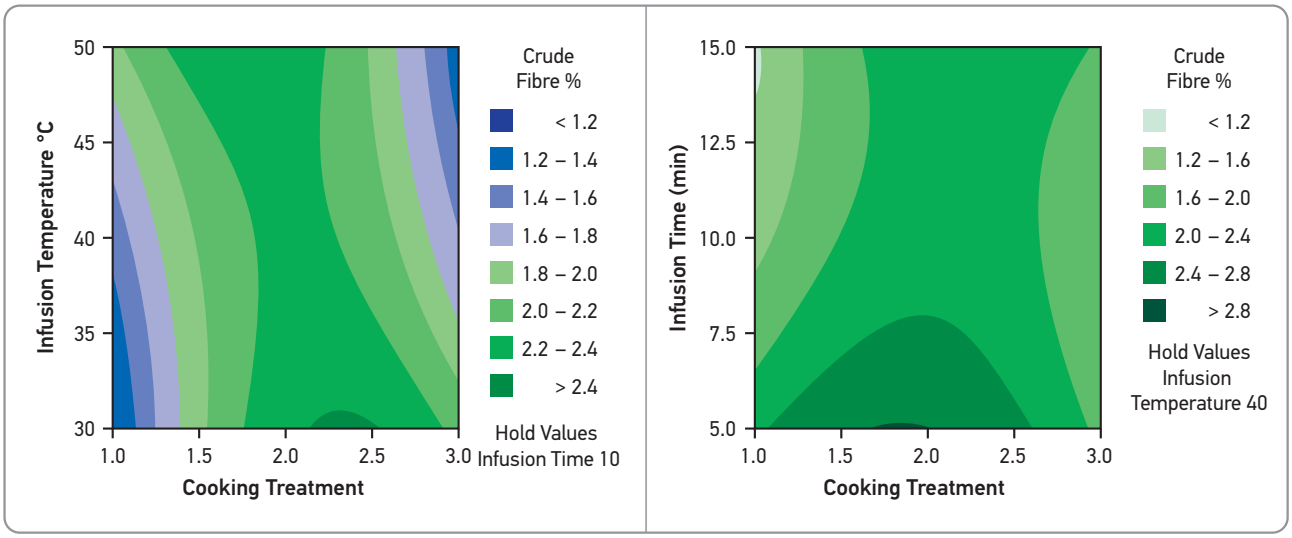

Figure 6. Effect of cooking treatment, infusion temperature and time on the crude fibre content of chicken *kilishi*

effect of cooking treatment, infusion temperature and time on the fibre content of chicken *kilishi.* The results obtained were higher than those obtained by *Soriano-Santos* (2010) and *Ogunmola et al.* (2013). The high content of crude fibre in the chicken *kilishi* could result from some of the ingredients used for the ingredient-mix preparation. *Ipinjolu et al.* (2004) reported ginger to have 24.5% of crude fibre, 5% in groundnut cake and traces of fibre content in pepper, clove and onion. The polynomial regression model in Equation 11 shows that the double interaction effect of infusion temperature, the interaction of infusion temperature and cooking treatment and interaction of infusion time and cooking treatment could lead to an increase in the crude fibre content, while a decrease in the cooking treatment, infusion time, double interaction effect of cooking treatment and double interaction effect of infusion time increased the crude fibre content. The R^2 and $R^2_{(adj)}$ for the crude fibre content of the polynomial regression model were 0.94 and 0.88 while the p-value and F-value were 0.000 and 16.79, respectively. This shows that the polynomial regression model is adequate to predict the effect of cooking treatment, infusion temperature and infusion time on the crude fibre content of chicken *Kilishi*. According to *Sanusi and Akinoso* (2021), R^2 that is greater than 0.8 shows a good fit to predict experimental results.

$$Crude\ fibre\ content = 0.578 - 3.852X_1 - \\ -0.408X_3 - 0.700X_1^2 + 0.001X_2^2 - 0.011X_3^2 + \\ + 0.04X_1X_2 + 0.062X_1X_3 \quad (10)$$

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – carbohydrate content

From Table 3, the carbohydrate content of the chicken *kilishi* ranged from 16.86 to 28.97%. Sample I, with the highest carbohydrate content (28.97%), was treated with steam and infused at 40°C for 15 min. Figure 7 shows the effect of cooking treatment, infusion temperature and time on the carbohydrate content of chicken *kilishi.* There were significant differences ($p \leq 0.05$) in the carbohydrate contents of the *kilishi*, which is a result of differences in the infusion temperature and time and the cooking treatment used. *Soriano-Santos* (2010) and *Emmanuel et al.* (2020) reported carbohydrate content of 2.1% and 2.3%, respectively, for chicken meat. However, the values obtained for this study were higher than those reported by *Soriano-Santos* (2010) and *Emmanuel et al.* (2020), and this could be attributed to the infusion of the chicken breast in the ingredient-mix for specific temperatures and times. The ingredients in the ingredient-mix formulation might be responsible for the high carbohydrate content.

The polynomial regression model in Equation 12 shows that an increase in cooking treatment, double interaction of infusion temperature and time, the interaction of infusion temperature and cooking treatment and interaction of infusion time and cooking treatment could lead to an increase in the carbohydrate content, while a decrease in the infusion temperature and time and double interaction effect of cooking treatment increases the carbohydrate content of chicken *kilishi*. The R^2 and $R^2_{(adj)}$ for

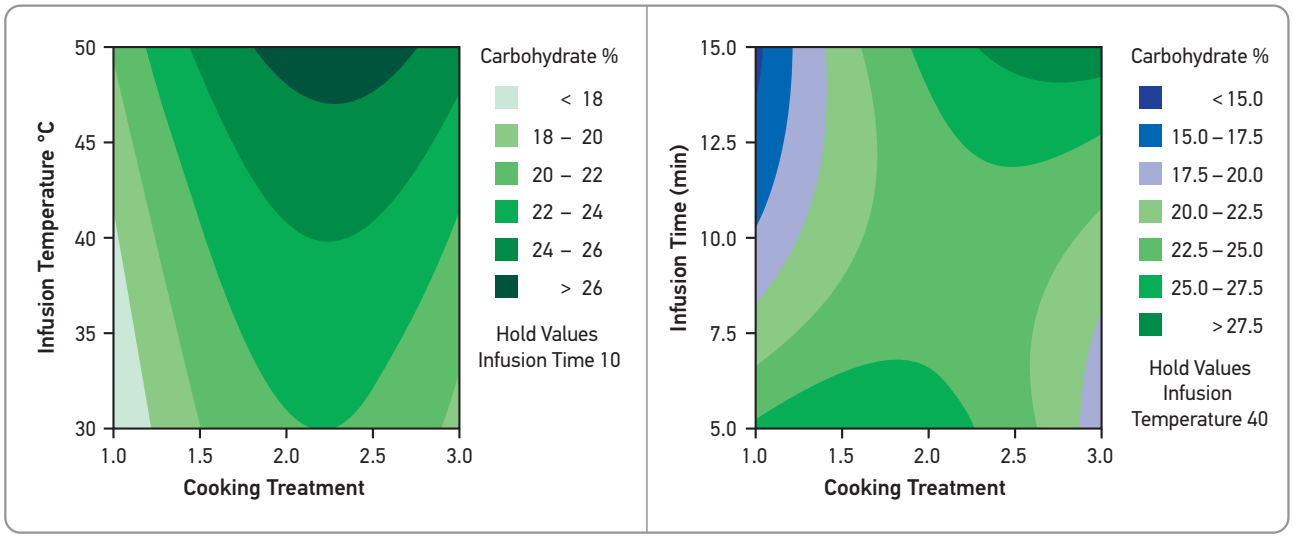

Figure 7. Effect of cooking treatment, infusion temperature and time on the carbohydrate content of chicken *kilishi*

the carbohydrate content of the polynomial regression model were 0.99 and 0.99 while the p-value and F-value were 0.000 and 1200.83, respectively. This shows that the polynomial regression model is adequate to predict the effect of cooking treatment, infusion temperature and infusion time on the carbohydrate content of chicken *kilishi*.

$$Carbohydrate\ content = 33.629 + 6.39X_1 - \\ - 0.161X_2 - 3.9X_3 - 4.140X_1^2 + 0.004X_2^2 + \\ + 0.087X_3^2 + 0.037X_1X_2 + 1.065X_1X_3 \quad (11)$$

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi –phosphorous content

The effect of cooking treatment, infusion temperature and infusion time on the phosphorous content of chicken *kilishi* is as shown in Figure 8. The phosphorous content of chicken *kilishi* samples ranged from 7.29 ppm to 12.07 ppm. Figure 9 shows the effect of cooking treatment, infusion temperature and time on the phosphorus content of chicken *kilishi*. Sample H had the highest phosphorous

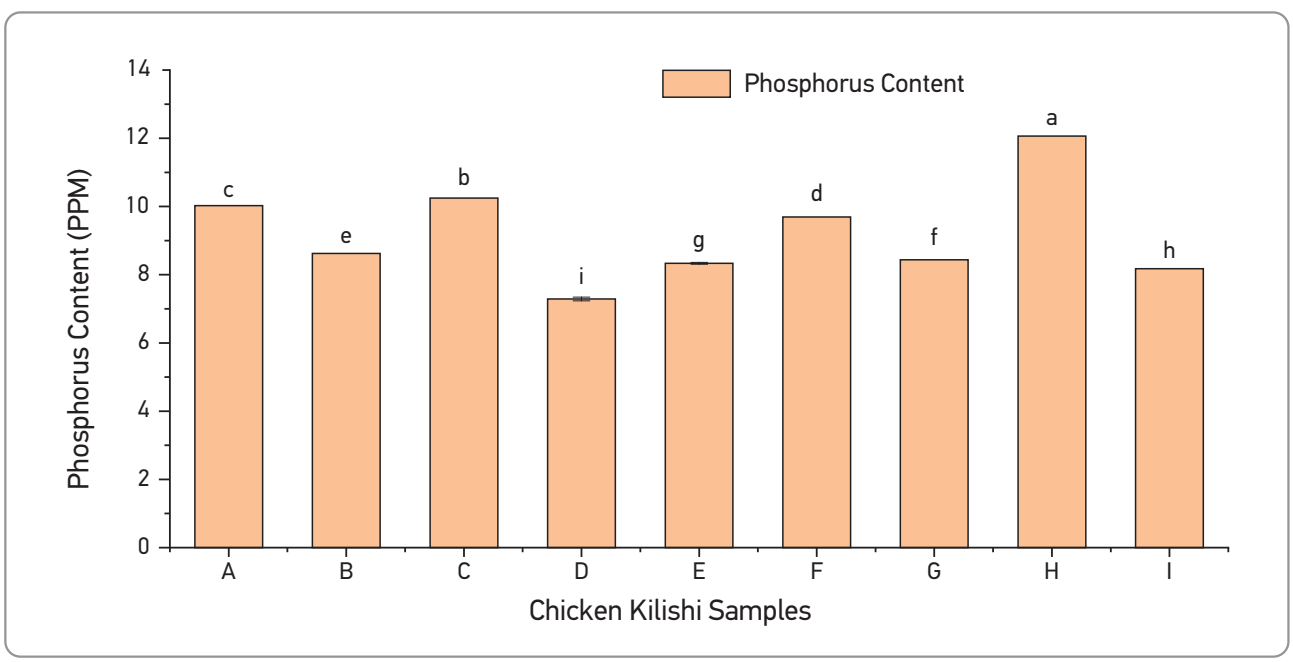

Figure 8. Effect of cooking treatment, infusion temperature and infusion time on the phosphorous content of chicken *kilishi*

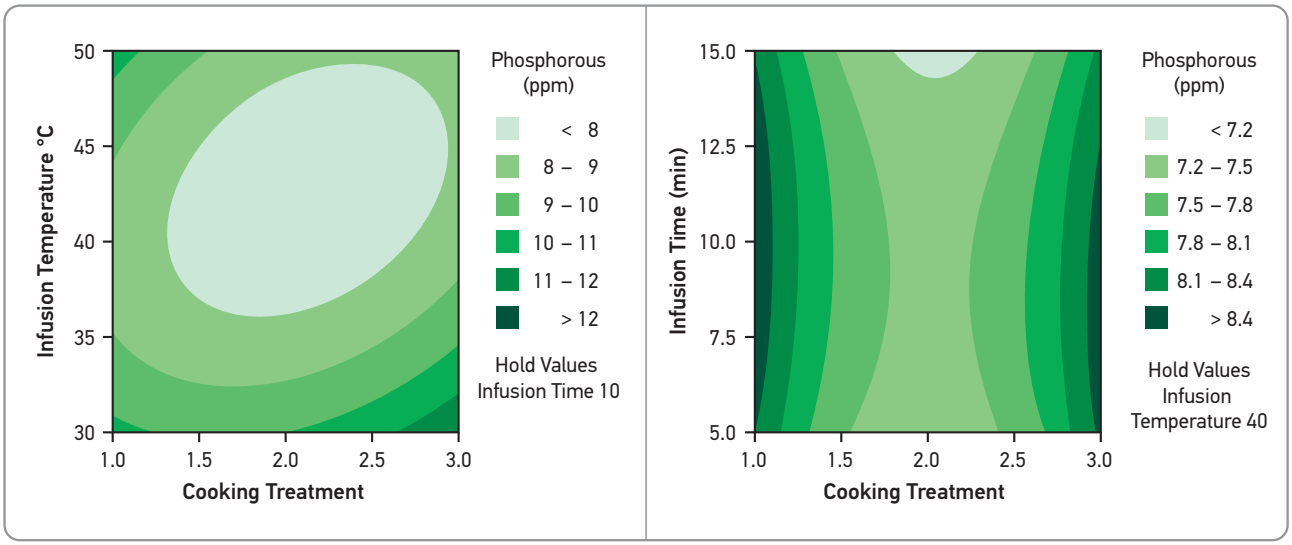

Figure 9. Effect of cooking treatment, infusion temperature and time on the phosphorus content of chicken *kilishi*

content (12.07 ppm), wherein the chicken breasts were steamed before drying and were infused at 30°C for 10 min, while the lowest phosphorous content (7.29 ppm) was observed in sample D, wherein the chicken breasts were steamed before drying and were infused at 40°C for 5 min. There was a significant difference at $p<0.05$ in the phosphorus content of the *kilishi*.

$$Phosporus\ content = 34.562 - 0.714X_1 - \\ - 1.28X_2 + 1.192X_3 + 1.158X_1^2 + 1.017X_2^2 - \\ - 0.009X_3^2 - 0.095X_1X_2 - 0.015X_1X_3 \quad (12)$$

The polynomial regression model in Equation 13 shows that an increase in infusion time, double interaction of cooking treatment, double interaction of infusion temperature, interaction of infusion time and cooking treatment could increase the phosphorous content, while decrease in the cooking treatment, infusion temperature and double interaction effect of infusion time and interaction of cooking treatment and infusion temperature increased the phosphorus content of chicken *kilishi*. *Livana et al.* (2018) reported that 69% of phosphorous could be leached into water within one minute, which is similar to our models that predict that phosphorus content should be higher in

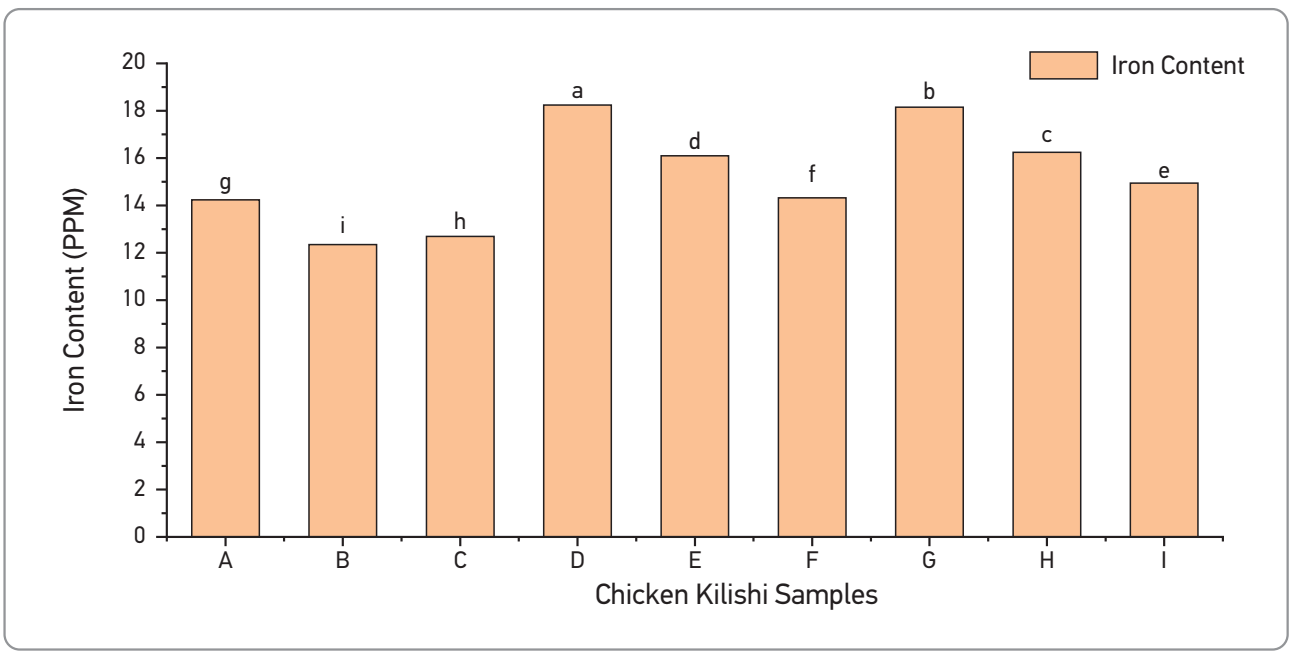

Figure 10. Effect of cooking treatment, infusion temperature and infusion time on the iron content of chicken *kilishi*

untreated *kilishi* than in the boiled and steamed *kilishi*. Moreover, the phosphorus content also decreased as infusion temperature increased because phosphorous solubility increases with temperature. The R^2 and $R^2_{(adj)}$ for the phosphorus content of the polynomial regression model were 0.99 and 0.99, while the p-value and F-value were 0.000 and 12065.73, respectively. This shows that the polynomial regression model is adequate to predict the effect of cooking treatment, infusion temperature and infusion time on the phosphorus content of chicken *kilishi*.

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – iron content

Figure 10 shows the effect of cooking treatment, infusion temperature and infusion time on the iron content of chicken *kilishi.* The iron content of chicken *kilishi* ranged from 12.35 ppm to 18.24 ppm. Sample D had the highest iron content (18.24 ppm), wherein the chicken breast was treated with boiling before drying and was infused at 40°C for 5 min, while the least iron content (12.35 ppm) was observed in sample B, wherein the chicken breasts were not treated before drying and were infused at 40°C for 10 min (Figure 11). There was a significant difference at $p \leq 0.05$ in the iron content of the *kilishi*. The iron content differed greatly across all *kilishi* due to different processing conditions and, most especially, the cooking treatment. According to *Inusa and Said* (2017), the iron content of different beef *kilishi* from a different location in Kano state, Nigeria had iron content ranging from 15.25 to 17.25 ppm, levels which were lower than the iron contents obtained in the *kilishi* snacks produced in our current study.

$$Iron\ content = 9.337 + 10.330X_1 + 0.049X_2 - \\ - 1.174X_3 - 1.45X_1^2 + 0.02X_2^2 + 0.037X_3^2 - \\ - 0.079X_1X_2 + 0.131X_1X_3 \quad (13)$$

The polynomial regression model in Equation 14 shows that an increase in cooking treatment, infusion temperature, the double interaction of infusion temperature and time and interaction of infusion time and cooking treatment increases the iron content, while a decrease in infusion time, the double interaction effect of cooking treatment and interaction of infusion temperature and cooking treatment increases the iron content of *kilishi*. The R^2 and $R^2_{(adj)}$ for the iron content of the polynomial regression model were 1.00 and 1.00, while the p-value and F-value were 0.000 and 1520171.18, respectively. This shows that the polynomial regression model can adequately predict the effect of cooking treatment, infusion temperature and infusion time on the iron content of chicken *kilishi*.

Effect of cooking treatment, infusion temperature and infusion time on proximate composition of chicken kilishi – zinc content

Figure 12 shows the effect of cooking treatment, infusion temperature and infusion time on the zinc content of chicken *kilishi.* The zinc contents of chicken *kilishi* ranged from 9.08 to 16.34 ppm. Sample C had the highest zinc content (12.07 ppm), wherein the chicken breast was untreated before drying and the ingredient-mix was infused at

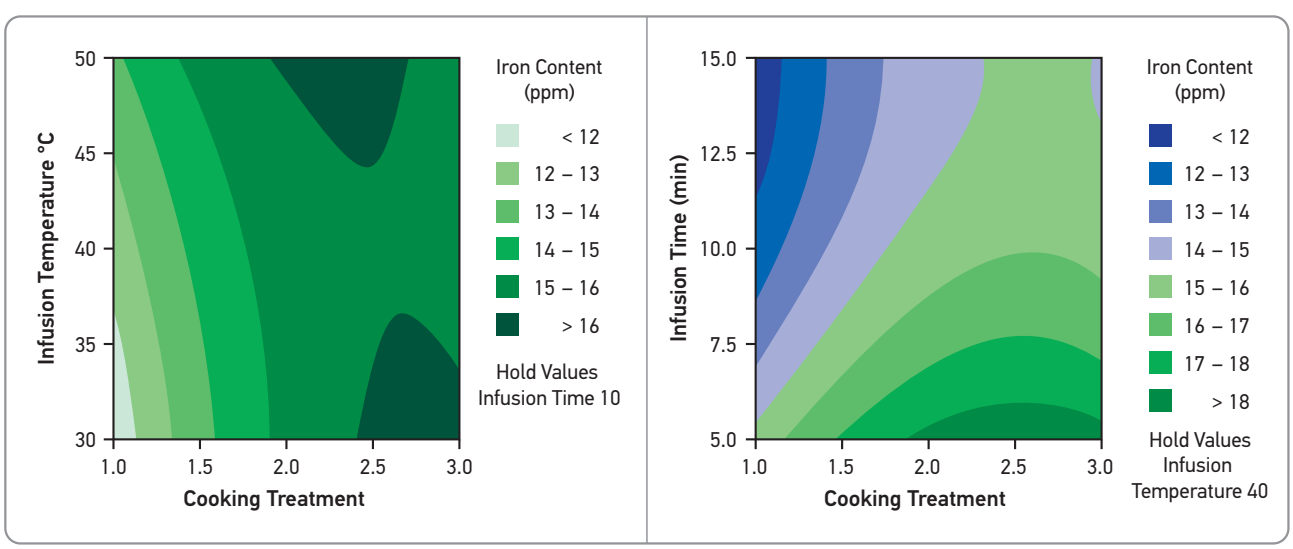

Figure 11. Effect of cooking treatment, infusion temperature and time on the iron content of chicken kilishi

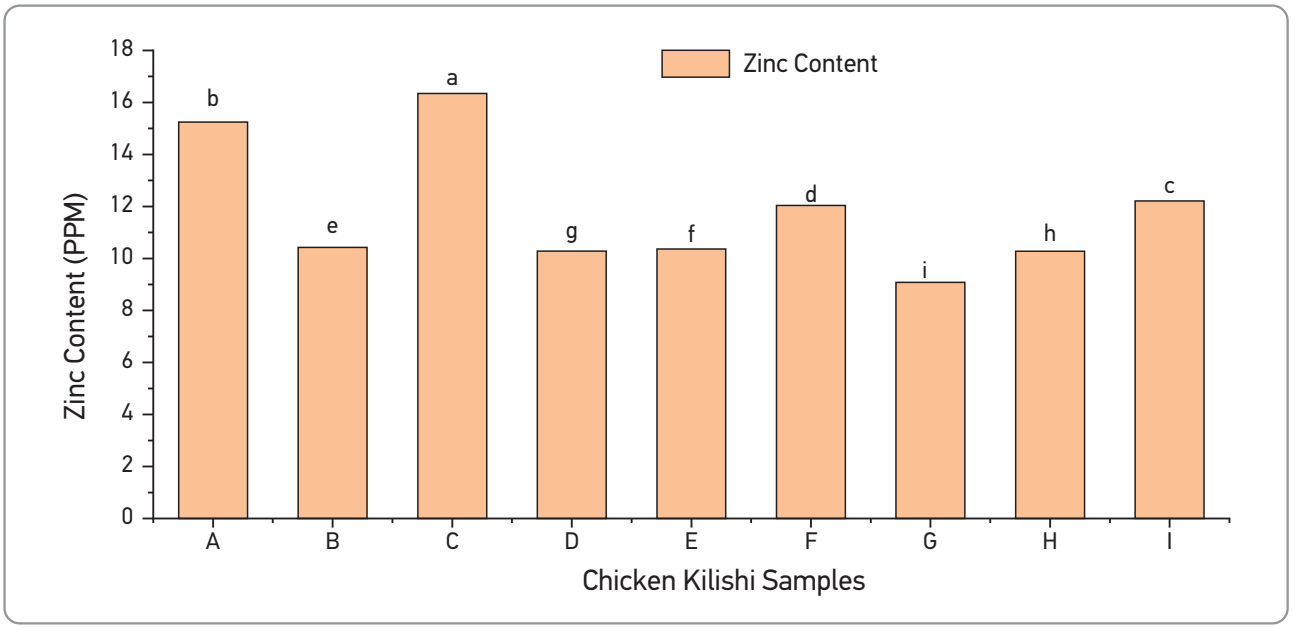

Figure 12. Effect of cooking treatment, infusion temperature and infusion time on the zinc content of chicken *kilishi*

50°C for 15 min, while the least zinc content (9.08 ppm) was observed in sample G wherein the chicken breast was steamed prior drying and the ingredient-mix was infused at 50°C for 5 min (Figure 13). There was a significant difference at $p<0.05$ in the zinc content of the *kilishi*. The differences in zinc content could be traced to the ingredient-mix infusion time. It was observed that zinc content increases as infusion time increases when the sliced chicken breast is steamed before drying. Therefore, the *kilishi* infused for 15 min had the highest zinc content. The zinc obtained in this study was higher than the 3.31 to 5 ppm reported by *Inusa and Said* (2017) for beef *kilishi*.

$$Zinc\ content = 56.718 - 4.259X_1 - 1.405X_2 - \\ - 3.273X_3 + 1.370X_1^2 + 0.023X_2^2 + 0.123X_3^2 - \\ - 0.1795X_1X_2 + 0.415X_1X_3 \quad (14)$$

The polynomial regression model in Equation 14 shows that an increase in the double interaction of cooking treatment, double interaction of infusion temperature and time and interaction of infusion time and cooking treatment increases the zinc content, while increase in the cooking treatment, infusion temperature and time and interaction of infusion temperature and cooking treatment decreases the zinc content of chicken *kilishi*. The zinc con-

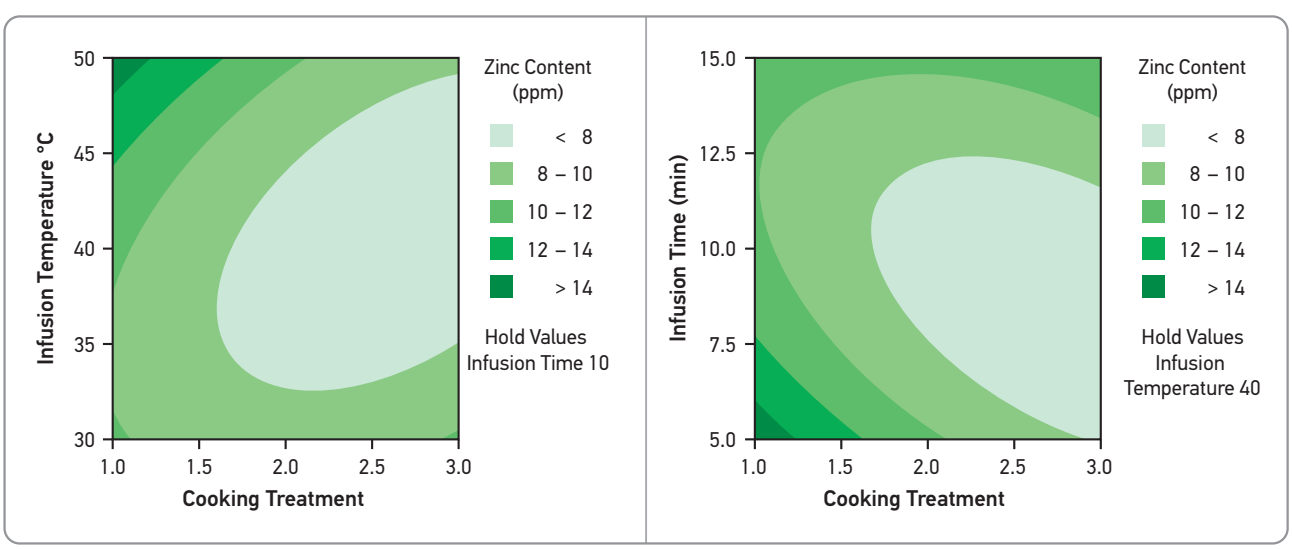

Figure 13. Effect of cooking treatment, infusion temperature and time on the zinc content of chicken *kilishi*

tent should decrease as cooking treatment increases, as reported by *Joyce et al.* (2018), due to leaching of the mineral into the cooking liquid. The R^2 and $R^2_{(adj)}$ for the zinc content of the polynomial regression model were 1.00 and 1.00, while the p-value and F-value were 0.000 and 1673234.40, respectively. This shows that the polynomial regression model can adequately predict the effect of cooking treatment, infusion temperature and infusion time on the zinc content of chicken *kilishi*.

Sensory attributes of chicken kilishi

Figure 14 shows the effect of cooking treatment, infusion temperature and infusion time on the sensory attributes of chicken *kilishi*. The panellists' scores for the appearance of the samples were within the range of 6.33 to 7.93. Sample C was rated as having the best appearance, while the appearance of sample E was rated the least liked. The preference for sample C could be because it did not undergo any cooking treatment. The panellists' scores for the taste of the samples were within the range of 6.33 to 7.67. Sample A was rated as giving the best taste, while the taste of sample E was rated as the least liked. The preference for samples A, B and C could be attributed to the lack of any cooking treatment applied to the chicken breast prior to drying. The panellists' scores for the flavour of the *kilishi* were within the range of 6.47 to 7.53. Sample A was rated as having the best flavour, while sample E was rated as having the least liked flavour. The panellists' scores for the spiciness of the *kilishi* were within the range of 6.40 to 6.93. Sample G gave the best spiciness, while sample B was rated as having the least liked spiciness. The panellists' scores for the texture of the *kilishi* were within the range of 5.27 to 7.20. Sample C gave the best texture, while sample E had the least liked texture. The overall acceptability of the chicken *Kilishi* samples produced was within the range of 6.07 to 7.73. Sample A had the highest overall acceptability score, while sample E was the least preferred. Therefore, Sample C was rated as having the best appearance and texture. Sample A was rated as having the best taste, flavour and overall acceptability, while sample G was rated as having the best spiciness. Therefore, it can be deduced that those chicken *kilishi* produced without any cooking treatment prior to drying were the most acceptable in terms of sensory attributes.

Optimisation of moisture content and protein of chicken kilishi

Figure 15 shows an optimisation plot for moisture content and protein content. The optimum process conditions were attained when the chicken breasts were not heat-treated and ingredient-mix infusion was conducted at approximately 41°C and for 6 min infusion time. The desirability values obtained for the moisture content and protein content were 1.00 and 0.69, respectively. The optimum protein content and moisture content was 48.12% and 5.34%, respectively. The composite desirability for achieving minimum moisture content and protein content was 0.84.

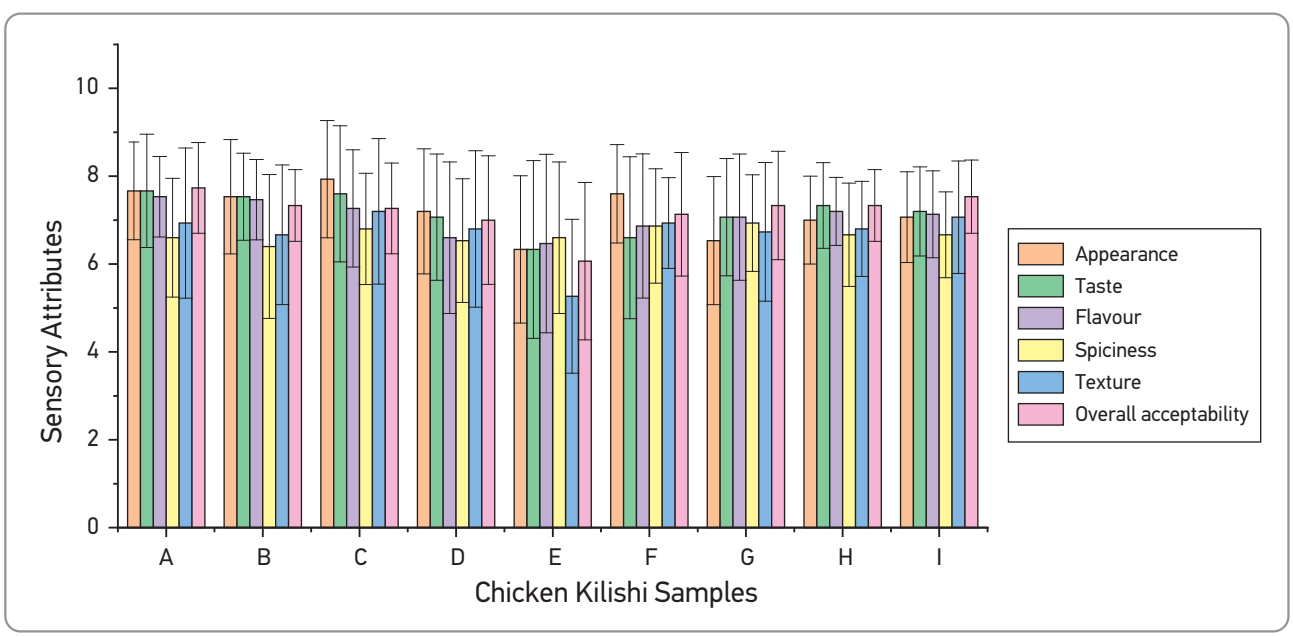

Figure 14. Effect of cooking treatment, infusion temperature and infusion time on the sensory attributes of chicken *kilishi*

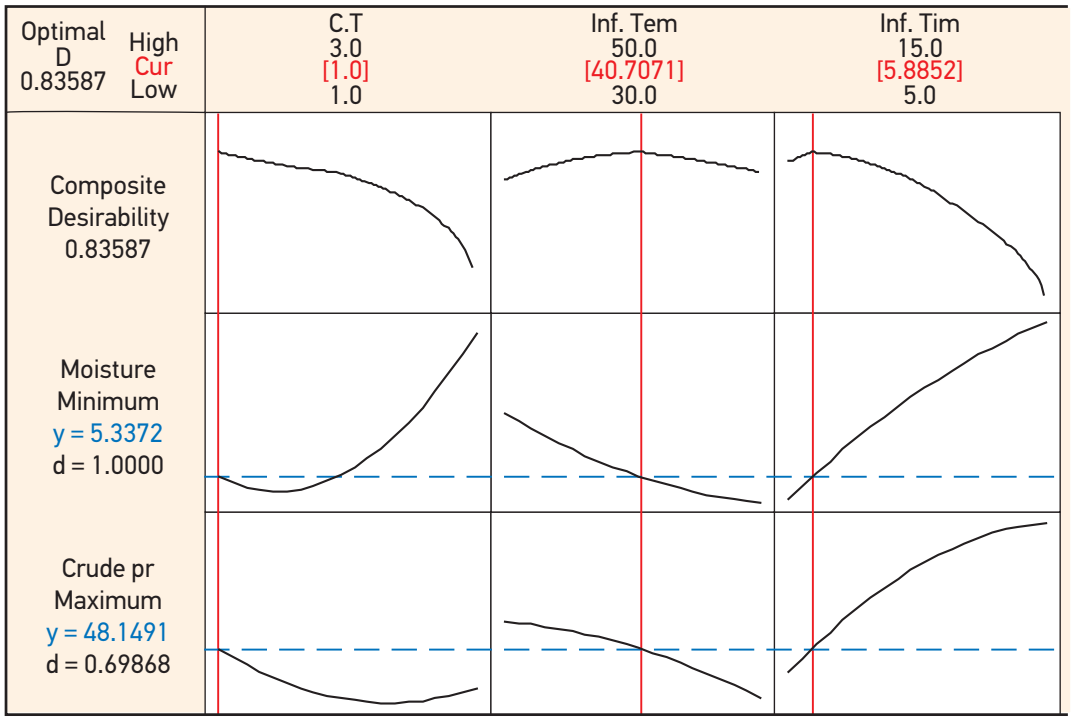

Figure 15. The optimum condition for the production of chicken *kilishi* with desirable moisture content and protein content. C.T is the cooking treatment, Inf. Temp. is the infusion temperature and Inf. Time is the infusion time.

Table 4. Validation of the optimum condition

Goal	Minimum values	Maximum values	Predicted optimum values	Desirability values	Experimental values	Error deviation (%)
Minimum moisture content (%)	5.34	11.81	5.34	1.00	5.55	3.93
Maximum protein content (%)	43.22	50.27	48.15	0.70	49.00	1.77

Sanusi and Akinoso (2021) reported that the closer the composite desirability to unity, the more reliable the proposed optimum condition. Therefore, according to Table 4, it can be adduced that there is a good agreement between the optimum predicted values and experimental values with minimum error deviation of 3.93 and 1.77% for minimum moisture content and maximum protein content, respectively. In addition, the crude fat, crude fibre, ash content, carbohydrate content, phosphorus content, zinc content and iron content obtained at optimum condition were 10.36%, 2.05%, 8.40%, 23.81%, 10.02 ppm, 15.24 ppm and 14.24 ppm, respectively. This validates the reliability of the proposed optimum condition for chicken *kilishi* with minimum moisture content and protein content.

Conclusion

Chicken *kilishi* was successfully produced under the influence of different cooking treatments, infusion temperatures and infusion times. The cooking treatment, ingredient-mix infusion temperature and infusion time significantly influence the proximate composition, phosphorus, iron and zinc content. The polynomial regression models were significant at $p<0.05$ and are capable of predicting the proximate and mineral composition of the ingredient-mix based dried chicken *kilishi* with R^2 and R^2_{adj} that range from 0.88 to 1.00. Chicken *kilishi* produced from untreated chicken breast produces *kilishi* with the most acceptable sensory attributes.

Modeliranje i optimizacija indikatora kvaliteta u proizvodnji sušenog pilećeg proizvoda na bazi mešavine sastojaka (pileći *kiliši*)

Mayowa S. Sanusi, Musliu O. Sunmonu, Ahmed O. Abdulkareem Abdulquadri Alaka

A p s t r a k t: Cilj ovog ispitivanja je bio da se istraži, modelira i optimizuje uticaj tretmana kuvanja (netretirano, kuvanje i tretman na pari), temperature infuzije mešavine sastojaka (30°C, 40°C i 50°C) i trajanja infuzije (5, 10 i 15 min) na indikatore kvaliteta sušenog pilećeg proizvoda (pileći kiliši) na bazi mešavine sastojaka od pilećih prsa. Indikatori kvaliteta (približan sastav i mineralni sadržaj) proizvedenog pilećeg kilišija su određeni i statistički analizirani korišćenjem hibridnog dizajna metodologije Taguchi-Response Surface. Proizvodnja pilećih kilišija od netretiranih pilećih prsa utiče na povećanje sadržaja pepela (9,04%), sadržaja sirovih proteina (50,28%) i sadržaja cinka (16,34 ppm). Međutim, pileći kiliši proizveden tretmanom na pari daje najveći sadržaj masti (14,02%), sadržaj ugljenih hidrata (28,97%) i sadržaj fosfora (12,07 ppm), dok kiliši podvrgnuti kuvanju imaju povećan sadržaj gvožđa (18,24 ppm), ali smanjen sadržaj vlage (5,32%). Razvijeni modeli polinomske regresije za indikatore kvaliteta bili su značajni sa R2 i R2adj koji se kreće od 0,88 do 1,00, respektivno. Optimalni uslovi procesa su postignuti kada pileća prsa nisu tretirana i infuzija mešavine sastojaka je sprovedena u trajanju od 6 minuta na 41°C.

Ključne reči: *pileća prsa; pileći kiliši; modeliranje; optimizacija; indikatori kvaliteta*

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Original scientific paper

Influence of phytobiotics in feed on the cost-effectiveness of broiler production during fattening

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A b s t r a c t: The aim of this study was to determine the effect of using phytobiotics in broiler feed on the economic efficiency parameters of fattening. The study was conducted on 240 broilers originating from a commercial incubator station, and the dietary trial was based on the group-control principle and lasted for 42 days (control group C — without the addition of phytogenic additives; experimental OI group — with the addition of phytogenic additive containing thymol and cinnamaldehyde, 100 g/t of food; experimental OII group — with the addition of phytogenic additive containing cumin, mint, cloves and anise, 150 g/t of food and; experimental OIII group — with the addition of phytogenic additive containing thymol, 750 g/t of food). The production results (body weight, average daily gain, feed conversion ratio) and economic efficiency parameters of broiler fattening were calculated for three intervals (1–10, 1–20 and 1–42 days). All production results in each interval were significantly better ($p<0.01$) in experimental broilers than in the control broilers. The best values of European factor of production efficiency and European broiler index were recorded in experimental groups that received feed with added phytobiotics (values were significantly higher, $p<0.01$, than in the control broilers). Also, the results obtained were compared with standard values for COBB 500 hybrids. The values obtained in this research were significantly lower ($p<0.05$) than standard values for Cobb 500. Analysing the data obtained from our study, the positive effects of including phytogenic additives in broiler feed mixtures were measured.

Keywords: *Cobb 500, production results, EBI, EPEF, antibiotic replacement.*

Introduction

On the economic side, in livestock (including poultry) production, economic viability is very important, and is affected by the feed composition and production results. For the production of fattening broilers, the world's major producers have their own nutrition guides (Cobb and Ross) and three feed mixtures are most often used, depending on the age of the chickens (starter, grower, finisher) (*Baltić et al*., 2011).

In recent years, as a consequence of the ban on antibiotics, various supplements, including phytobiotics, have been used to preserve animal health and obtain good production results. Increasing attention in animal nutrition is focused on phytogenic additives (phytobiotics) as possible acceptable alternatives to antibiotics. The use of phytogenic additives in poultry nutrition achieves similar effects as the use of antibiotics, but they do not leave residues or have withdrawal periods, and they could become ideal feed additives and successfully replace antibiotics as growth promoters in food (*Glamočlija et al*., 2016; Šević, 2016).

Poultry is a very profitable branch of livestock production, because in a relatively short time, with little investment, large quantities of high quality products can be produced for which there is a constant demand on the market and which are very easy to sell (*Basić and Grujić*, 2013). In addition to meeting market needs, broiler meat production is especially interested in the economic viability of broiler fattening. In recent years, two indices have been used to calculate the cost-effectiveness of fattening: the European factor of production efficiency (EPEF) and the European broiler index (EBI). EPEF is used worldwide as an indicator of broiler growth performance (*Aviagen*, 2019; *Van*, 2003; *Susim et al*., 2020). Some authors, in addition to EPEF, use EBI, which can be calculated for flocks of different ages, to assess the performance of broilers (*Van*, 2003; *Marcu et al*., 2013; *Cengiz et al*., 2019). EPEF is a tool for measuring the growth performance of

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broilers (*Aviagen*, 2019; *Van*, 2003). Therefore, the factors involved in the EPEF are body weight gain (BWG), feed coversion ratio (FCR) and viability and are considered universal measures for evaluating broiler performance (*Marcu et al*., 2013).

In some countries, the EBI is used for measuring broiler growth performance, calculated for flocks with different slaughter ages. In this case, the factors involved in calculating the EBI are average daily gain (ADG), feed conversion ratio (FCR) and viability. EBI values are always lower than EPEF, because in the ADG calculation, the chicks' weight to one day is excluded (*Van*, 2003). Higher EPEF or EBI values indicate better fattening economics (*Marcu et al*., 2013; *Lukić et al*., 2020).

The aim of this study was to determine the effect of using phytobiotics in broiler diets on the economic parameters of fattening.

Materials and Methods

The study was conducted on a broiler farm in Srbac (45.0989° N, 17.5217° E), Republika Srpska (Bosnia and Herzegovina), with broilers originating from a commercial incubator station. The dietary trial was based on the group-control principle and lasted for 42 days. One-day-old Cobb 500 chickens of both sexes were used, and females and males had an average body weight of 46.33±3.57 g and 46.93±3.83 g, respectively. The study was conducted on a total of 240 broilers divided into four groups of 60 animals housed in groups of 10 birds per pen in six repetitions (control group C — without the addition of phytogenic additives; experimental OI chickens — with the addition of phytogenic additive containing thymol and cinnamaldehyde, 100 g/t of food; experimental OII chickens — with the addition of phytogenic additive containing cumin, mint, cloves and anise, 150 g/t of food, and; experimental OIII chickens — with the addition of phytogenic additive containing thymol, 750 g/t of food). The study was divided into three phases. The first phase lasted 0–10 days, the second phase 11–20 and the third phase 21–42 days. Conditions in the facility (ventilation, heating, lighting and relative humidity) were according to the technological standards and recommendations for this hybrid (*NRC*, 1994; *Cobb-Vantress*, 2018a; *Cobb-Vantress*, 2018b). Pens were bedded with straw and broilers provided with fresh water and feed *ad libitum*.

During the study, the broilers were fed with complete mixtures for fattening chickens that contained standard raw materials and chemical composition. Three mixtures were used (Table 1) that fully met the needs of broilers at different phases of fattening (*Cobb-Vantress*, 2018a). A complete mixture for feeding OI chickens (starter) was used from 0–10 days, then a complete mixture for feeding OII chickens (grower) was used from 11–20 days and a complete mixture for feeding OIII chickens (finisher) was used from 21–42 days. The broiler feed consisted mainly of corn, wheat, soy, minerals, amino acids and premixes. The average contents of protein, moisture, cellulose, fat and ash in the broiler feed mixtures are shown in Table 1. Data in Table 1 show the feed mixtures used for broiler fattening in the age groups were of standard chemical composition and fully satisfied the needs of broilers in all fattening phases.

The main task of the study was to determine the impact of broiler diets with feed mixtures containing different phytogenic additives on production results and yield parameters, and determine if the use of natural growth stimulants in intensive broiler farming is justified from an economic point of view.

Table 1. Raw material composition of broiler feed mixtures used in fattening, mean % ± standard deviation

Mixture (age of chickens in days)	Moisture	Ash	Protein	Fat	Cellulose
Starter (0–10)	8.04±0.24	5.45±0.14	24.98±0.57	6.09±0.37	2.04±0.05
Grower (11–21)	9.38±0.09	4.88±0.13	22.17±0.21	7.03±0.26	2.16±0.04
Finisher (22–42)	9.98±0.07	4.76±0.21	20.91±0.87	5.44±0.11	2.38±0.26

Therefore, minimal corrections were made to the mixtures in order to achieve the desired goal. The control group of broilers was fed a mixture without phytogenic additives, while the experimental groups received feed with phytogenic additives.

During the study, the health status of broilers and production results (body weight, weight gain, feed conversion) were monitored, and mortality was recorded. At the beginning and end of each phase of the study (starter, grower, finisher), the body weight of each individual animal and pen feed consumption were measured and complete feed mixtures were analysed, then other production results were calculated from the obtained data. The economic efficiencies of broiler production during fattening were calculated as EPEF (*Baltić et al*., 2011; *Van*, 2003) and EBI (*Van*, 2003). The following formulas were used to calculate these indicators:

BWG (g) for the period = BW (g) at the end of period − BW (g) on first day

$$\textbf{ADG}\ (\text{g/chick/d}) = \frac{\text{BWG (g)}}{\text{number of days in the growth period}}$$

$$\textbf{FCR}\ (\text{kg feed/kg gain}) = \frac{\text{Cumulative feed intake (kg)}}{\text{total weight gain (kg)}}$$

Viability (%) = number of broilers at the end of each fattening period (%)

$$\textbf{EPEF} = \frac{\text{Viability (\%)} \times \text{BW (kg)}}{\textit{age}\ \text{(d)} \times \text{FCR (kg feed/kg gain)}} \times 100$$

$$\textbf{EBI} = \frac{\text{Viability (\%)} \times \text{ADG (g/chick/d)}}{\text{FCR (kg feed/kg gain)} \times 10}$$

Statistical analysis

The results obtained were compared by statistical analysis using Microsoft Excel 2010 and GraphPad Prism software, version 8.00 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). To determine the significance of the differences between the examined groups of compared parameters, the analysis of variance (ANOVA) was used. Testing of the significance of the difference between the arithmetic means of the compared parameters and the standard values (i.e. the recommendations for this hybrid (*Cobb-Vantress*, 2018a)) was conducted according to *Petz et al*. (2012). Differences were considered significant if $p<0.01$ or $p<0.05$ were observed.

Results and Discussion

Table 2 shows the production results of broilers during fattening, as well as the calculated economic efficiency parameters of broiler fattening. On day 10 of fattening, a significant difference ($p<0.05$) was found between the control and experimental groups of broilers. The same significant difference was established on day 21 and at the end of fattening. The control broilers had a significantly lower ($p<0.05$ — day 21; $p<0.01$ — day 42) body weight compared to the experimental broiler groups. The ADG of broilers during fattening was calculated for three intervals, i.e. from 1 to 10 days, from 1 to 21 days and 1 to 42 days. In all three intervals, the ADG of control broilers was significantly ($p<0.01$) lower than those of the experimental groups. Also, on day 10, the ADG of OII broilers was significantly lower ($p<0.01$) than that of the other experimental groups. At the end of broiler fattening, significant differences in ADG were found between the experimental groups of broilers ($p<0.05$; $p<0.01$).

FCR is shown (Table 2) for the individual fattening intervals. Control broilers produced the worst FCR in all fattening intervals, in relation to experimental groups of broilers. On day 10, the FCR of control and OIII broilers differed significantly ($p<0.05$). Observed for the whole study, the best FCR was achieved by OI broilers (1.85), followed by OIII (1.855) and OII (1.89) broilers.

Production efficiency was assessed using EBI and EPEF. The best EPEF and EBI in this study were recorded in broilers that received feed with added phytobiotics. These economic parameters were significantly higher ($p<0.01$) in the experimental broiler groups than in the control broiler group, although significant differences ($p<0.01$) were found between the experimental groups (Table 2).

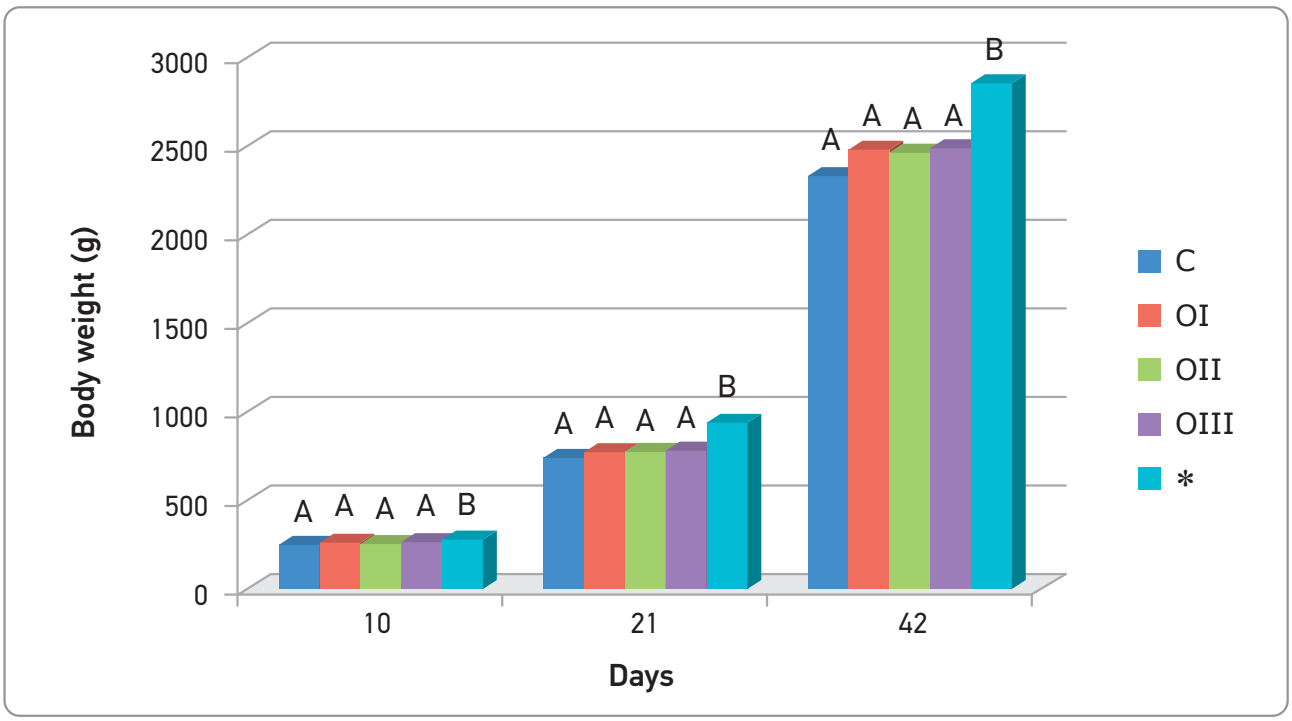
The results obtained were compared with standard values for COBB 500 hybrids. Figures 1 and 2 show broiler weight and FCR, respectively, after different fattening intervals (1–10 days; 1–21 days, and 1–42 days). The values obtained in this research were significantly lower ($p<0.05$) than the standard weight and FCR for Cobb 500 broilers after 42 days' fattening.

The realized EPEF and EBI values (Figures 3 and 4, respectively), cumulative indicators of the final result and success of fattening, were significantly higher in experimental broilers than in the control broilers ($p<0.05$), while the values obtained for broilers in this study were significantly lower than standard values for Cobb 500 broilers.

Table 2. Production results and economic efficiency parameters during broiler fattening

Fattening interval, days	Parameters	Control	OI Broilers	OII Broilers	OIII Broilers
1to 10	BW (kg)	$0.254^{abc}\pm0.017$	$0.265^{a}\pm0.021$	$0.257^{b}\pm0.024$	$0.266^{c}\pm0.028$
	ADG (g)	$20.78^{ABC}0.149$	$21.79^{AD}\pm0.098$	$21.10^{BDE}\pm0.110$	$21.96^{CE}\pm0.080$
	FCR (kg feed/kg gain)	$2.16^{a}\pm0.108$	1.96±0.158	1.99±0.123	$1.94^{a}\pm0.111$
	Viability (%)	98	99	99	100
	EPEF	$115.69^{ABC}\pm1.435$	$133.85^{ADa}\pm2.642$	$127.85^{BDE}\pm2.007$	$137.11^{CEa}\pm1.309$
	EBI	$94.29^{ABC}\pm3.032$	$110.05^{AD}\pm2.448$	$104.99^{BDE}\pm1.831$	$113.21^{CE}\pm1.472$
1 to 21	BW (kg)	$0.744^{abc}\pm0.082$	$0.778^{a}\pm0.040$	$0.778^{b}\pm0.044$	$0.784^{c}\pm0.063$
	ADG (g)	$33.20^{ABC}\pm0.242$	$34.84^{A}\pm0.172$	$34.86^{B}\pm0.104$	$35.11^{C}\pm0.323$
	FCR (kg feed/kg gain)	1.89±0.142	1.73±0.135	1.77±0.167	1.76±0.193
	Viability (%)	98	98	98	99
	EPEF	$183.70^{ABC}\pm3.493$	$209.87^{A}\pm2.817$	$205.12^{Ba}\pm2.116$	$210.00^{Ca}\pm2.991$
	EBI	$172.17^{ABC}\pm2.55$	$197.34^{A}\pm3.22$	$193.00^{B}\pm2.06$	$197.49^{C}\pm3.70$
1 to 42	BW (kg)	$2.334^{ABC}\pm0.148$	$2.485^{A}\pm0.218$	$2.461^{B}\pm0.191$	$2.489^{C}\pm0.210$
	ADG (g)	$54.46^{ABC}\pm0.323$	$58.06^{Aa}\pm0.104$	$57.49^{BDa}\pm0.349$	$58.16^{CD}\pm0.351$
	FCR (kg feed/kg gain)	2.07±0.12	1.85±0.199	1.89±0.103	1.85±0.193
	Viability (%)	98	98	98	99
	EPEF	$263.09^{ABC}\pm2.106$	$313.42^{ADa}\pm2.033$	$303.83^{BDE}\pm1.984$	$317.13^{CEa}\pm2.724$
	EBI	$257.85^{ABC}\pm2.44$	$307.54^{ADa}\pm1.51$	$298.10^{BDa}\pm3.01$	$311.21^{CE}\pm1.65$

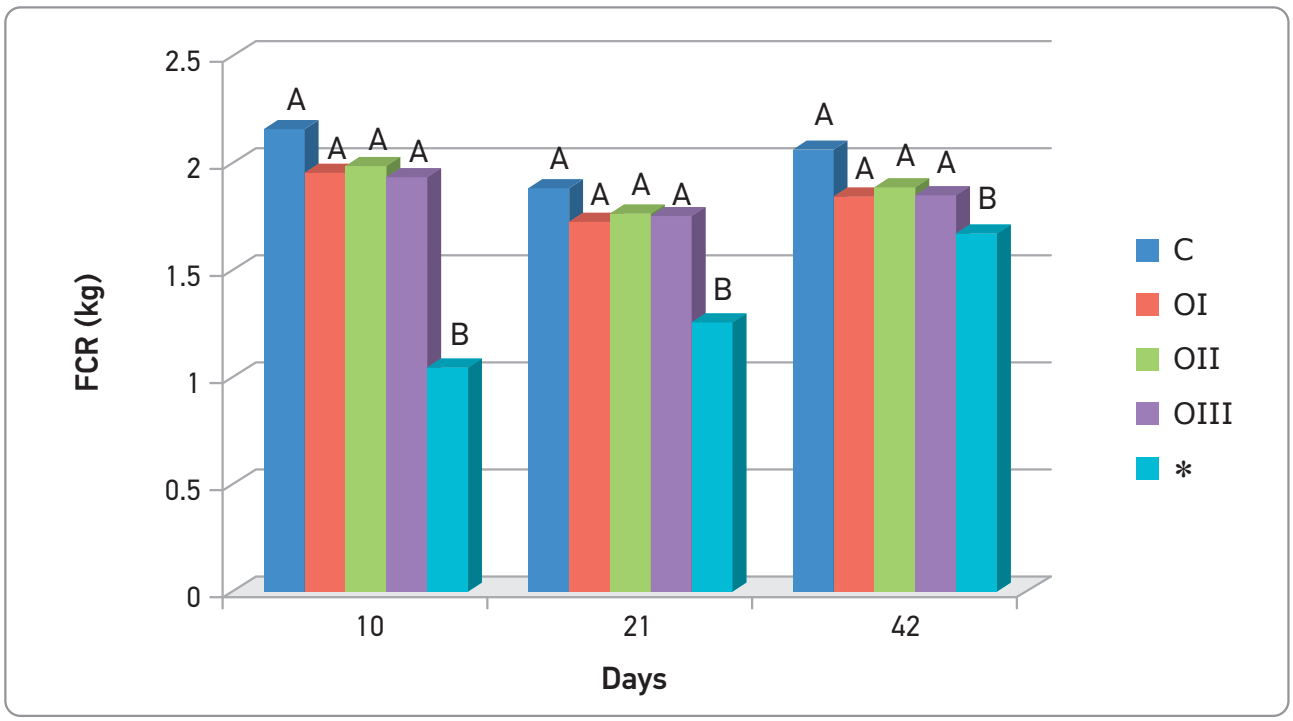
Legend: BW – body weight; ADG – average daily gain; FCR – feed conversion ratio; EPEF - European Production Efficiency Factor; EBI – European Broiler Index; Same letter in a row ^{a,b,c} $p<0.05$; ^{A,B,C,D,E} $p<0.01$.



Legend: * According to the Cobb 500 broiler guide; Different letters $^{A, B}$ indicate body weight differs ($p<0.05$)

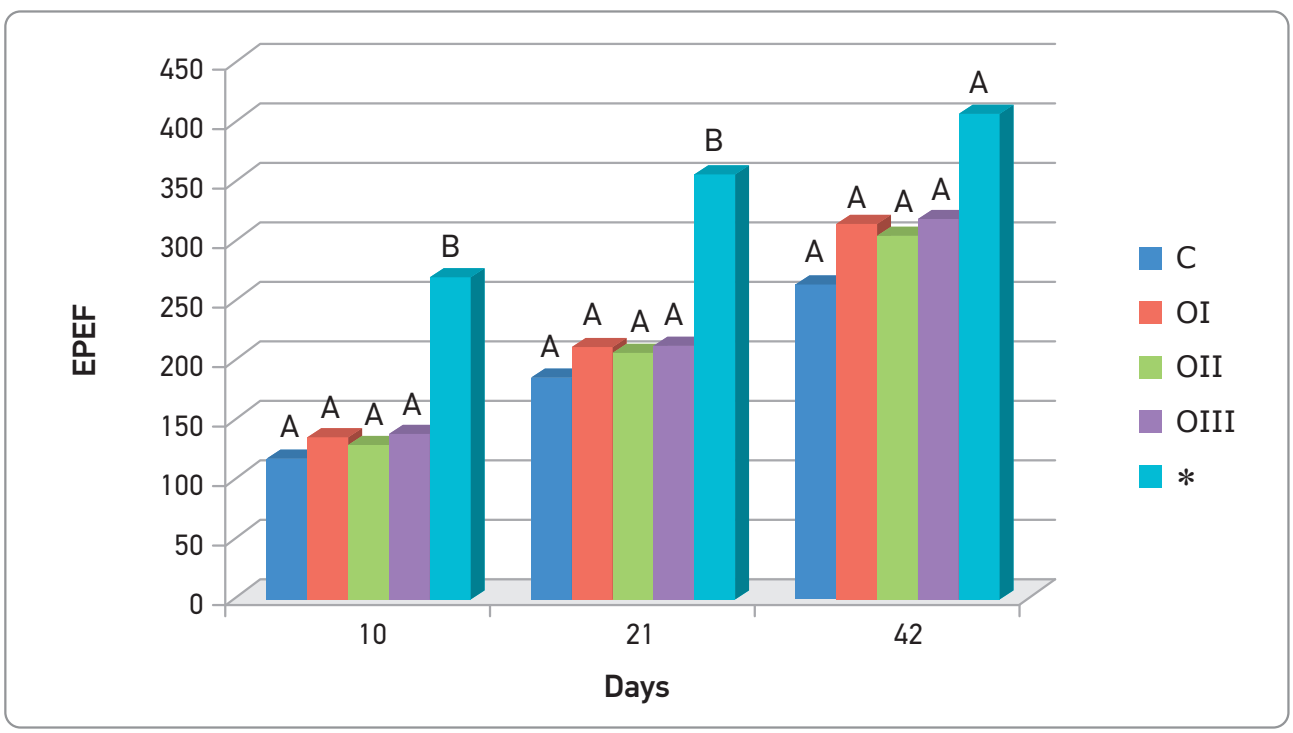
Figure 1. Average broiler weights (g) compared with standard weights for Cobb 500 broilers

Based on the production results (body weight, ADG, FCR etc.), it is difficult to talk about the economic viability of production, but rather, this can be better assessed on the basis of economic parameters. The production results obtained within this research were in accordance with the results of other authors (Šević, 2016; *Branković Lazić et al*., 2021; *Baltić et al*., 2018; *Milanković et al*., 2019). However, anal-



Legend: *According to the Cobb 500 broiler guide; Different letters $^{A, B}$ indicate feed conversion ratio differs ($p<0.05$)

Figure 2. Average feed conversion ratio (kg) of broilers compared with standard values for Cobb 500 broilers



Legend: *According to the Cobb 500 broiler guide; Different letters $^{A, B}$ indicate different EPEF ($p<0.05$)

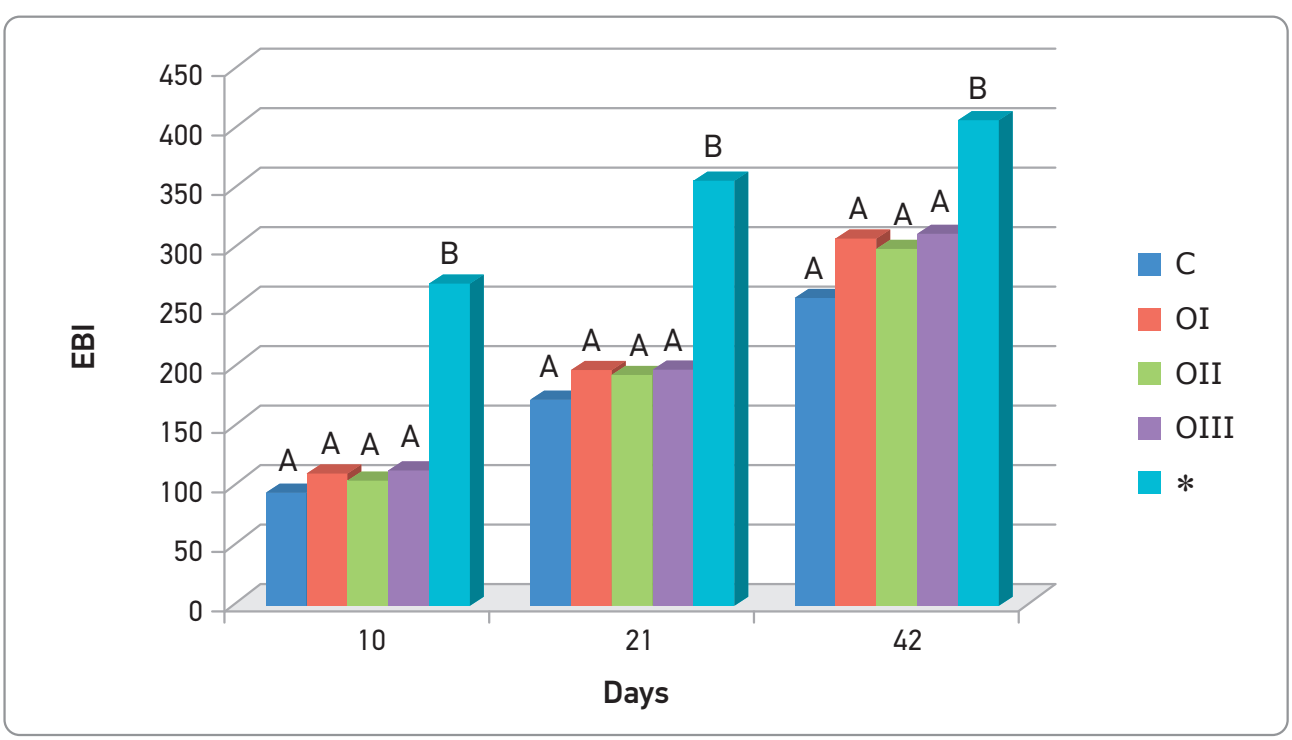
Figure 3. Average European factor of production efficiency (EPEF) for broiler groups compared with standard values for COBB 500 broilers

ysis of performance data (body weight, ADG, FCR and mortality) are essential to calculate the economic efficiency of broiler growth.

The profitability and cost-effectiveness of chicken meat fattening and marketing have been examined by various authors (*Hamra*, 2010; *Rhodes et al*., 2008; *Szollosi et al*., 2014). They emphasized that when calculating profitability and economics, it is necessary to know the selling prices of chicken meat and business costs. Costs are divided into fixed and variable. Variable costs vary according to the level of total activity or volume. Fixed costs do not change in relation to changes in volume or changes in the level of total activity. *Rhodes et al.* (2008) emphasize that broiler breeders must calculate variable and fixed costs when calculating profitability and economics. Variable costs include the costs of concentrate mixtures, electricity, cleaning, ongoing maintenance of facilities and equipment, telephones and alarms. Fixed costs include the costs of day-old chicks, labour, insurance, taxes and land use charges (real estate tax). With good management practice, a grower can reduce costs, which is a condition for increasing profits. The main feature of newer cost reduction strategies is less reliance on statistical sources of cost reduction (such as economies of scale or effects of experience) and increasing reliance on continuous improvement, innovation, restructuring, business process redesign and rigorous analysis of production activities (*Milićević*, 2003). *Salihbašić et al.* (2014) point out that the following types of costs occur within the costs of chicken meat production: one-day chickens, concentrate mixtures (starter, grower, finisher), immunoprophylaxis, cooperation in fattening chickens and dead and discarded chickens.

Investing in improving the welfare of broilers affects the cost of fattening. Calculating the cost of animal welfare is a complex task. Some animal welfare measures increase production costs, but this can be offset by higher quality products or lower losses due to reduced disease or injury. There are ways to improve animal welfare that do not compromise productivity and are not necessarily expensive. It is important to investigate the economic and social impact of animal friendly measures on production and production alternatives, in order to reconcile animal welfare and economic imperatives (*Hansen*, 2002; *Dawkins et al*., 2004; *Blandford*, 2006; *Bessei*, 2006; *Utnik-Banaś et al*., 2014). Improving animal welfare can lead to reduced disease and mortality, as well as reduced disease control and treatment expenditures (*Hansen*, 2002; *Dawkins et al*., 2004; *Blandford*, 2006).

Of the total costs of fattening chickens, the costs of concentrate mixtures account for about 70%. Efficient use of feed has the greatest impact on managing production costs. The basic parameters used to



Legend: * According to the Cobb 500 broiler guide; Different letters $^{A, B}$ indicate different EBI ($p<0.05$)

Figure 4. European Broiler Index (EBI) values of broilers compared with standard values for COBB 500 broilers

measure economy and profitability are outputs, revenues and expenditures. Effects are equally considered to be material products and services derived from the production process of the organization (*Utnik-Banaś et al*., 2014; *Tesić and Nedić*, 2015). Profit is the difference between the value of production (total income) of fattening and the cost of fattening and is determined at the end of fattening. The profit of fattening chickens, expressed in the simplest form, is the value of the final product less the input costs caused by the production of that product.

The use of phytobiotics in broiler diets in this study has produced better production results and better economic viability parameters (EPEF, EBI). Analysing the data obtained from our study, the positive effects of adding phytogenic additives to broiler feed mixtures were measured. Phytobiotics added to broiler feed had a positive impact on all measured production results. Broilers that received phytobiotics had higher body weight and total weight gain, lower feed consumption and better feed conversion than broilers that did not consume phytobiotics.

Conclusion

The results in this study support the use of phytobiotics in broiler diets, since this is economically justified given the good production results (broiler weight at the end of fattening, feed conversion, average daily gain, growth). Therefore, the use of phytobiotic preparations in broiler feed had a positive effect on increasing the economic viability parameters of broiler production. There is not much published research that has monitored the impact of different types of phytobiotics on the economic viability parameters (EPEF, EBI), so these and similar experiments open up numerous opportunities for further research.

Phytobiotics in poultry feed could become ideal feed additives and successfully replace antibiotics as growth promoters in broiler feed.

Ispitivanje uticaja delovanja fitobiotika u hrani na ekonomičnost proizvodnje brojlera u tovu

Jelena Janjić, Kristina Šević Savić, Radmila Marković, Dragan Šefer, Drago Nedić, Spomenka Đurić, Branislav Vejnović, Milorad Mirilović

A p s t r a k t: Cilj ovog istraživanja bio je da se utvrdi uticaj upotrebe fitobiotika u ishrani brojlera na ekonomske parametre tova. Eksperiment je sproveden na ukupno 240 brojlera poreklom iz komercijalne inkubatorske stanice, zasnovan na grupno-kontrolnom sistemu i trajao je 42 dana (kontrolna grupa K — bez dodatka fitogenih aditiva, ogledna OI grupa — sa dodatkom fitogenog aditiva koji sadrži timol i cinamaldehid, 100 g/t hrane, ogledna OII grupa - uz dodatak fitogenog aditiva koji sadrži kim, nanu, karanfilić i anis, 150 g/t hrane, i ogledna OIII grupa - uz dodatak fitogenog aditiva koji sadrži timol, 750 g/t hrane). Proizvodni rezultati (telesna masa, prosečni dnevni prirast, konverzija hrane) i parametri ekonomske efikasnosti tova brojlera su izračunati u tri perioda (od 0. do 10. dana; od 11. do 20. i od 21. do 42. dana). Svi proizvodni rezultati u svakom periodu bili su statistički bolji ($p<0,01$) u eksperimentalnim grupama nego u kontrolnoj grupi. Najbolje vrednosti EPEF i EBI u ovom istraživanju zabeležene su u eksperimentalnim grupama (značajno veće, $p<0,01$, nego u kontrolnoj grupi) koje su dobijale hranu sa dodatkom fitobiotika. Takođe, dobijeni rezultati su upoređeni sa standardnim vrednostima za hibride Cobb 500. Vrednosti dobijene u ovom istraživanju bile su značajno manje ($p<0,05$) od standardnih vrednosti za Cobb 500. Analizirajući podatke dobijene iz našeg eksperimenta, uočava se pozitivan efekat dodavanja fitogenih dodataka krmnim smešama za brojlere.

Ključne reči: *Cobb 500, rezultati proizvodnje, EBI, EPEF, zamena antibiotika.*

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Original scientific paper

Quality and acceptance of ready-to-cook dishes prepared with fillet and belly flap area of hybrid sorubim

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Abstract: Ready-to-cook dishes were prepared using the fillet and the belly flap area of hybrid sorubim. The chemical, microbiological and sensorial characteristics of the obtained products were evaluated. Four treatments were elaborated: (T1) fillet, tomato sauce and vegetables; (T2) fillet without tomato sauce or vegetables; (T3) belly flap area with added tomato and vegetables and; (T4) belly flap area without tomato sauce or vegetables. After marinating by immersion using brine, fish cuts with and without sauce/vegetables were placed in vacuum packages and cooked. Microbiological and proximate analyses showed the products were within the standards required by current legislation. Sensory analysis showed acceptance rates above 70%. The dishes prepared with fillet and belly flap area cuts with added tomato sauce and vegetables were the most acceptable according to the sensory evaluation and purchase intention, with no difference between the types of cut. Therefore, the use of the less commercially desirable meat cut (belly flap area) is feasible in the elaboration of the ready-to-cook moqueca dishes.

Keywords: *moqueca, fish product, ready-to-cook, food safety, Pseudoplatystoma reticulatum, Pseudoplatystoma corruscans.*

Introduction

Fish contain extremely important components for the human diet, including high quality proteins, lipids and bioactive components. The presence of polyunsaturated fatty acids in fish is attributed to confer numerous benefits to human health, including the reduction of the risk of coronary heart diseases, hypertension and diabetes, and in the prevention of certain cardiac arrhythmias and sudden death (*Ramos Filho et al.,* 2008; *Cavenaghi-Altemio et al.,* 2013; *Menegazzo et al.,* 2014).

In order to take advantage of these benefits, the fish industry provides a wide variety of consumer products, such as whole fish, fillets, slices and pulp, dried, chilled or frozen, salted, breaded or canned, as well as other treatments. However, filleted fish is the product preferred by consumers (*Silva et al.,* 2018).

Hybrid sorubim (*Pseudoplatystoma* sp.) is one of the most important native carnivorous species of great potential for aquaculture in Brazil (*Honorato et al.,* 2015). The meat is widely accepted due to its excellent palatability and absence of intramuscular spines (*Hisano et al.,* 2013). Most of the commercialised hybrid sorubim comes from the Pantanal and Amazon regions, and is consumed in the Southeast, South and Midwest regions (*Crepaldi et al.,* 2006).

In consumer food markets, sensoriality and pleasure, health and well-being, convenience and practicality, reliability and quality, and sustainability and ethics are attractive matters that promote competitiveness of the industries worldwide. Thus, ready-to-eat and semi-ready-to-eat dishes, in small portions, have had a great increase in demand (*Siekierski et al.,* 2013; *Incoronato et al.,* 2016).

Ready- or semi-ready-to-eat dishes containing products of animal origin contain processed or partially processed products including meat of different species of slaughterhouse animals and/or meat products, and/or any other product of animal origin prepared separately or combined with ingredients such as sauces, vegetables, flour and cereals, subjected to a suitable technological process (*MAPA,* 2001). They can be classified as traditional, continental, ethnic, vegetarian, and low-calorie. These products have culinary or recipe competences added to them by manufacturers that result in a high degree of read-

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iness, completion, and commodity. They are divided into five categories: canned, ambient, frozen, chilled, and dry (*Harris & Shiptsova*, 2007).

In Brazil, moqueca is a spicy stew of seafood, vegetables and palm oil, with different seasonings added, and is much appreciated by the fish-eating population. It originates from the fisheries developed by the Portuguese, to the products of which black slaves added their usual ingredients of African cuisine when they arrived in Brazil (*Watkins*, 2015).

The aims of this work were to develop ready-to-cook dishes using the fillet and belly flap area cuts of hybrid sorubim and to evaluate the chemical, microbiological and sensorial characteristics of the obtained products.

Materials and Methods

Cuts of hybrid sorubim

Twenty hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*) fishes were obtained from a local fish farm. They were transported to the Laboratory of Food Technology from the Federal University of Grande Dourados, Dourados, MS, Brazil, where they were slaughtered and filleted into two cuts (fillet and belly flap area), under refrigerated conditions. Each fish weighed on average 800 g, being 400 g of fillet and 150 g of belly. Thus, 8 individuals were utilised to obtain the total amount of fillet and 20 individuals were utilised to obtain the total amount of belly to carry out all the analyses (approximately 1,500 g per treatment). Figure 1 shows the body locations of the cuts used in the preparation of the ready-made dishes. The belly flap area has less commercial value than does the fillet.

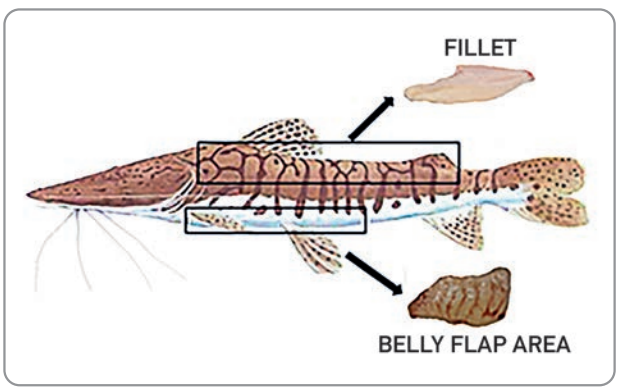

Figure 1. Body locations of the fillet and the belly flap area in hybrid sorubim (*Pseudoplatystoma reticulatum* x *Pseudoplatystoma corruscans*) fishes.

Ready-made dishes of hybrid sorubim

Four treatments were prepared with the fillet and belly flap area of hybrid sorubim. Treatment 1 (T1) was elaborated with fillet, tomato sauce and vegetables (onion, and red, yellow and green peppers); Treatment 2 (T2) with fillet without tomato sauce or vegetables; Treatment 3 (T3) with belly flap area added with tomato sauce and vegetables (onion, and red, yellow and green peppers) and Treatment 4 (T4) belly flap area without tomato sauce or vegetables. The fillets and belly flap areas of the four treatments were marinated at 4°C during 12 h by immersion using brine (1:1) of the following composition (in %): water, 95.82; sodium chloride, 3.2, citric acid, 0.4; dehydrated garlic, 0.4; and white pepper powder, 0.2. After marinating, the steaks and belly flap areas of the treatments were placed in vacuum (polyethylene and nylon) packages. For treatments T1 and T3, 50% of sauce plus vegetables in relation to the weight of the fish was also added. All treatments were sealed in a vacuum Sealer (Tecmaq model AP 450). The moqueca sauce was prepared with 60% industrial tomato sauce, 35% coconut milk, 3% refined salt and 2% palm oil. Pasteurisation was then carried out in a water bath (Quimis model Q334M-28) at 85°C up to 72°C of internal temperature of the fish. After that, thermal ice-shock was carried out and the products were immediately stored at −18°C to avoid the formation of microcrystals.

Chemical analysis

Moisture, crude protein, and crude ash contents of the ready-made dishes were determined in triplicate according to the methods described by *AOAC* (2012). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46B), protein by the Kjeldahl method (method 928.08) and ash by using the muffle oven technique (method 920.153). The lipid content was obtained in triplicate by the extraction method with cold organic solvent (*Bligh & Dyer 1959*). The carbohydrate content was estimated by difference.

Microbiological analysis

Microbiological analyses of the raw materials and ready-made dishes were performed for thermo-tolerant coliforms at 45°C, *Staphylococcus aureus* and *Salmonella* spp. in accordance with the methodology described elsewhere (*USDA/FSIS, 1998*).

Sensory analysis

Sensory analyses of the ready-made dishes were conducted by 48 non-trained panellists. A nine-point hedonic scale (9=like extremely; 1=dislike extremely) was used for evaluation of the attributes colour, odour, texture and taste. The treatments were heated in microwave ovens until the internal temperature reached 60°C, then they were cut into 2.0 × 2.0 × 1.5 cm cuboids and served in disposable containers kept warm for no longer than 15 min., coded with three-digit random numbers. Overall acceptation was evaluated in terms of purchase intention using a 5-point scale, where 5 = certainly would purchase, 4 = probably would purchase, 3 = perhaps would purchase / perhaps would not purchase, 2 = probably would not purchase and 1 = certainly would not purchase, which was expressed as the percentage of total score. For frequency of consumption of fish and fish moqueca, a 4-point scale was utilised, where 4 = weekly, 3 = 2 to 3 times a week, 2 = 2 to 3 times a month and 1 = annually (*Cavenaghi-Altemio et al,* 2018). The acceptation index (AI) was calculated according to the following equation: AI = (average of the attributed grades / maximum attributed grade) x 100. The sample was considered acceptable if the value was greater than 70% (*Stone & Sidel*, 2004).

Statistical analysis

Results were evaluated through analysis of variance (ANOVA) and Tukey's test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 8.0. The purchase intention and the consumption frequencies were analysed as percentages.

Results and Discussion

Table 1 shows the results of the proximal composition analysis carried out for the ready-to-eat dishes prepared with fillet and belly flap area cuts of hybrid sorubim. Moisture content varied from 75.91 to 83.43%, with a significant difference ($p < 0.05$) between the treatments, except between treatments T1 and T3, with sauce in the formulation. These two treatments had the highest moisture contents (Table 1), which were very similar to the 83.07% found in the study with silver catfish (*Rhamdia quelen*), steamed, and canned with tomato sauce (*Cozer et al.,* 2014).

Ash content ranged from 1.40 to 2.53%, with no significant difference between treatments ($p>0.05$). The literature reports ash content of 2.00% for silver catfish in tomato sauce (*Cozer et al.,* 2014). This value is very similar to that found in this study (Table 1). However, these same authors found only 0.49% of ash in silver catfish fillet with tomato sauce, which is probably due to the differences between the raw materials.

There was a significant difference ($p<0.05$) between the treatments in relation to the protein content, except between T2 and T4, corresponding to fillet and the belly flap area, both without sauce/vegetables, respectively. These treatments (T2 and T4) contained the highest protein contents, 17.50 and 17.68%, respectively (Table 1). The lower protein content in treatments with sauce/vegetables (T1 and T3) than in treatments without sauce/vegetables (T2 and T4) is easily explained by the composition of the sauce, which contains sugar, xanthan gum and modified starch, among other carbohydrates.

A protein content of 17.90% was previously reported for sorubim (*P. corruscans*) (*Ramos Filho et al.*, 2008). This value is very close to those obtained for T2 and T4, which did not have added

Table 1. Proximate composition of ready-made dishes prepared from the fillet and belly flap area cuts of hybrid sorubim

Treatment	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	Carbohydrates (%)
T1	$83.32^{a} \pm 0.21$	$8.85^{a} \pm 0.67$	$2.12^{a} \pm 0.04$	$1.66^{a} \pm 0.12$	4.05
T2	$79.88^{b} \pm 0.42$	$17.50^{b} \pm 0.40$	$0.92^{b} \pm 0.03$	$1.40^{a} \pm 0.30$	0.30
T3	$83.43^{a} \pm 0.67$	$5.30^{c} \pm 0.45$	$5.05^{c} \pm 0.10$	$2.53^{a} \pm 0.13$	3.69
T4	$75.91^{c} \pm 0.71$	$17.68^{b} \pm 0.89$	$2.95^{d} \pm 0.04$	$2.19^{a} \pm 0.65$	1.27

Legend: Means with the same letter in the same column do not differ statistically at 5% (P>0.05). Treatment 1 (T1) was elaborated with fillet, tomato sauce and vegetables (onion, and red, yellow and green peppers); Treatment 2 (T2) with fillet without tomato sauce or vegetables; Treatment 3 (T3) with belly flap area with tomato sauce and vegetables (onion, and red, yellow and green peppers), and Treatment 4 (T4) with belly flap area without tomato sauce or vegetables.

Table 2. Microbiological analyses of hybrid sorubim and ready-made dishes prepared from the fillet and belly flap area cuts of hybrid sorubim

Treatment	Microbiological analyses		
	Coliforms at 45ºC	*Staphylococcus aureus*	*Salmonella* spp.
Raw fish	<1.0 x 10^1 CFU/g est.	<1.0 x 10^1 CFU/g est.	Absent in 25 g
T1	<1.0 x 10^1 CFU/g est.	<1.0 x 10^1 CFU/g est.	Absent in 25 g
T2	<1.0 x 10^1 CFU/g est.	<1.0 x 10^1 CFU/g est.	Absent in 25 g
T3	<1.0 x 10^1 CFU/g est.	<1.0 x 10^1 CFU/g est.	Absent in 25 g
T4	<1.0 x 10^1 CFU/g est.	<1.0 x 10^1 CFU/g est.	Absent in 25 g

Legend: CFU: Colony forming units. Treatments (T1, T2, T3, and T4) according to Table 1.

sauce/vegetables (Table 1). Values close to those obtained in treatments T1 and T3 (with added sauce/vegetables) were reported for flitch and silver catfish fillets, both with added tomato sauce (*Cozer et al.,* 2014). The small differences were due to different fish and preparation methods.

The lipid content significantly differed ($p<0.05$) between all treatments, with the highest value (5.05%) found in T3 (belly flap area with sauce/vegetables) and the lowest value (0.92%) in T2 (fillet without sauce/vegetables). The lipid content of the treatments with sauce was higher than the treatments without sauce/vegetables (Table 1) due to the inclusion of palm oil and coconut milk in the sauce.

Carbohydrates are present in minimum amounts in fishes. Thus, variations in carbohydrate contents are mainly related to the addition of tomato sauce and vegetables (onion, and red, yellow and green peppers) containing carbohydrates. This was evident when comparing T1 and T3 (fillet and belly flap area with sauce/vegetables, respectively) with T2 and T4 (fillet and belly flap area without sauce/vegetables, respectively) (Table 1).

Table 2 shows the results of the microbiological analyses carried out for the hybrid sorubim *in natura* and for the ready-to-cook dishes prepared with the steamed cuts (fillets and belly flap areas) of the hybrid sorubim. The results showed that all products were within the parameters required by current Brazilian legislation.

For raw fish, cooled or frozen, that will not be consumed raw, the established parameters are the absence of *Salmonella* sp. in 25 g and a maximum count of $5x10^3$ CFU/g for *Staphylococcus aureus*. For precooked fish, cooled or frozen, the established parameters are: absence of *Salmonella* sp. in 25 g, and maximum counts of 5×10^2 CFU/g for *S. aureus*, and of $1x10^2$ CFU/g for coliforms at 45°C (*ANVISA*, 2001). Thus, the procedures performed during the handling and preparation of the products were adequate from the hygienic-sanitary stand-point for the safety of the sensory panellists. Therefore, sensory evaluations were conducted.

Table 3 presents the results for the acceptance test of the colour, taste, texture and odour sensory attributes of the ready-to-cook dishes prepared with fillets and belly flap areas of hybrid sorubim. The averages of attribute scores ranged from the equivalent of "I did not like" to "I liked moderately" for treatments that did not contain sauce, both for fillets (T2) and belly flap areas (T4). For the treatments with sauce (T1 and

Table 3. Sensory analysis of ready-made dishes prepared from the fillet and belly flap area cuts of hybrid sorubim

Attribute	Treatment			
	T1	T2	T3	T4
Door	$8.08^a \pm 0.71$ (89.77)	$6.69^b \pm 1.69$ (74.33)	$8.08^a \pm 0.79$ (89.77)	$6.13^b \pm 1.70$ (68.11)
Colour	$8.10^a \pm 0.90$ (90.00)	$6.67^b \pm 1.60$ (74.11)	$8.08^a \pm 0.71$ (89.77)	$6.50^b \pm 1.50$ (72.22)
Taste	$8.46^a \pm 0.65$ (94.00)	$7.15^b \pm 1.64$ (79.44)	$8.21^a \pm 0.99$ (91.22)	$6.94^b \pm 1.56$ (77.11)
Texture	$8.42^a \pm 0.71$ (93.55)	$7.42^b \pm 1.47$ (82.44)	$8.31^a \pm 0.72$ (92.33)	$7.06^b \pm 1.34$ (78.44)

T3), these averages were higher and varied from the equivalent of "I liked" to "I liked it very much".

Means with the same letter in the same column do not differ statistically at 5% (P>0.05). Values in parenthesis are the acceptability index (%). Treatments (T1, T2, T3, and T4) are according to Table 1.

The average scores for each of the sensorial attributes evaluated did not significantly differ (p>0.05) between T1 and T3, or between T2 and T4. However, there were significant differences between T1 and T2, as well as between T3 and T4 (p<0.05) (Table 3). Thus, despite them utilising different cuts, neither the treatments with sauce (fillet and belly flap area) nor the treatments without sauce (fillet and belly flap area) had detectably different sensory attributes. Considering that the belly flap area is a cut of lesser interest and commercial value, it may be an option for commercial ready-to-cook moqueca (T1 and T3) dishes.

Table 3 also shows (in parenthesis) the AIs for the odour, colour, flavour and texture sensory attributes of the ready-to-cook dishes prepared with fillets and belly flap areas of hybrid sorubim. Products with AI above 70% were considered acceptable (*Stone & Sidel*, 2004). All the sensorial attributes of the evaluated treatments were acceptable, except for the odour attribute of T4, which scored an AI of 68.11%. The other AIs ranged from 89.77 to 94.00% for ready-to-cook moqueca dishes prepared with fillets and belly flap areas, and from 72.22 to 82.44% for dishes prepared with belly flap areas without sauce. In this sense, the best AIs were obtained for the treatments with added sauce (Table 3).

Figure 2 shows the percentage of the purchase intention frequencies of the ready-to-cook dishes prepared with fillets and belly flap areas of hybrid sorubim. The sums of the purchase intentions, "certainly would buy" and "probably would buy", were 93.75, 50.00, 95.85 and 35.40% for T1, T2, T3, and T4, respectively, showing that the ready-to-cook moqueca dishes (T3 and T1) both received higher percentages of positive purchase intention than did the fish without sauce (Figure 2). Comparing the results of Table 3 with those of Figure 2, it was clear that the panellists' purchase intentions did not reflect the acceptance rate of the ready-to-cook dishes without sauce. However, despite the lower purchase intention, the AIs (Table 3) demonstrated that it is possible to prepare ready-to-cook moqueca dishes from fillets and belly flap areas without the addition of sauce.

Figure 3A presents percentages of fish consumption frequency, while Figure 3B presents the percentages of fish moqueca consumption frequency as reported by our panellists. The highest percentage of fish consumption frequency and fish moqueca consumption frequency was 2 to 3 times a month. If compared to the other animal proteins, the frequency is very low. This may be due to both the price of the fish and the preparation time of this dish. On the other hand, these data indicate that there is a large market for this type of dish in Brazil.

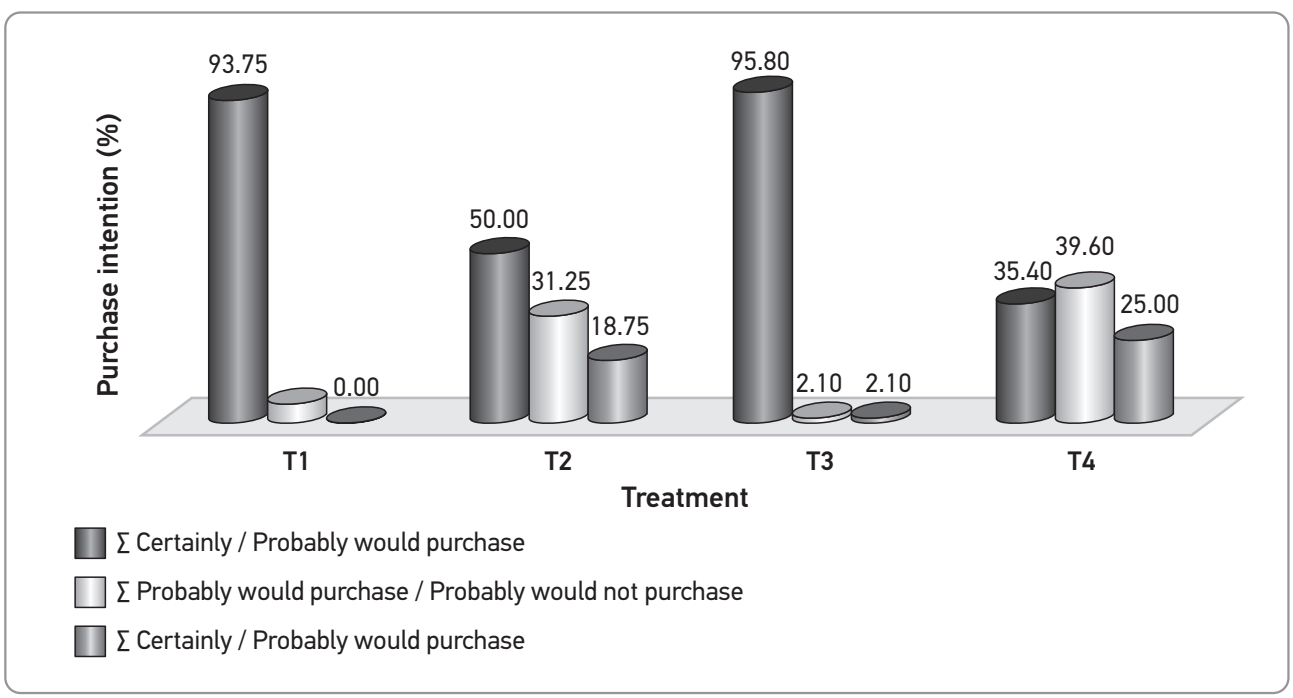

Figure 2. Purchase intention (%) of ready-made dishes prepared from the fillet and belly flap area cuts of hybrid sorubim. Treatments (T1, T2, T3, and T4)) according to Table 1.

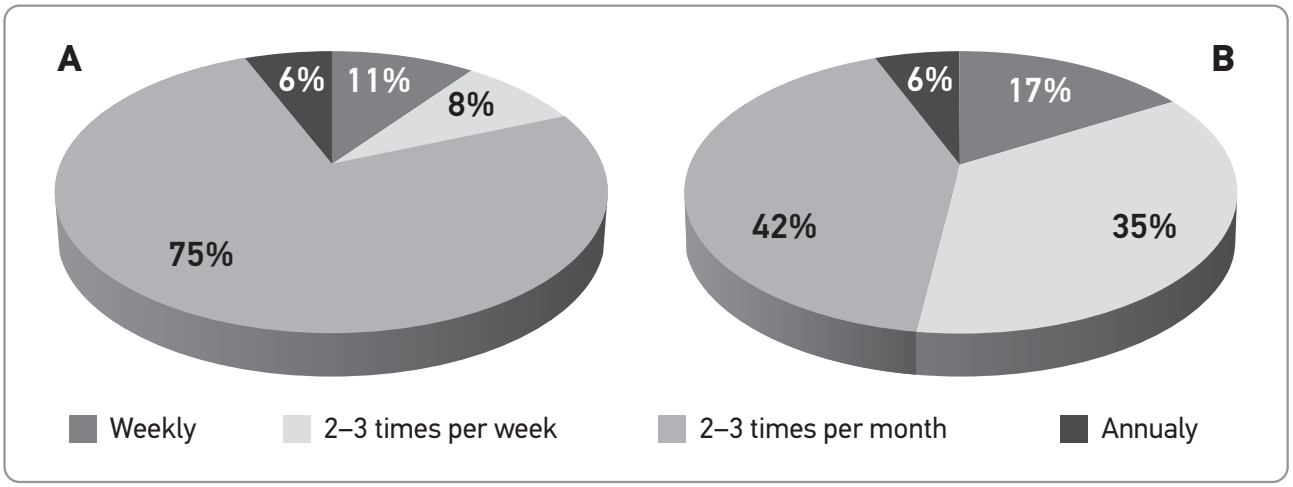

Figure 3. Frequency of consumption (%) of fish (A) and fish moqueca (B).

Conclusions

All treatments complied with the legislation for chemical and microbiological parameters, as well as the definition of ready-to-cook products of animal origin. The dishes prepared with fillet and belly flap area cuts added with tomato sauce and vegetables were more acceptable to the panellists according to their sensory evaluation and purchase intentions, with no difference between the types of cut. Therefore, the use of a less commercially desirable meat cut (belly flap area) is feasible in the elaboration of ready-to-cook moqueca dishes.

Kvalitet i prihvatljivost gotovih jela pripremljenih od fileta i područja trbušnog operkuluma/režnja hibridnog sorubima

Angela Dulce Cavenaghi-Altemio, Dandrea Sakie Matsumori, Jéssica Lima de Menezes, Gustavo Graciano Fonseca

A p s t r a k t: Gotova jela za kuvanje su pripremljena korišćenjem fileta i područja trbušnog operkuluma/režnja hibridnog sorubima. U ovoj studiji, ocenjene su hemijske, mikrobiološke i senzorne karakteristike dobijenih proizvoda. Četiri različita tretmana su postavljena: (T1) file, paradajz sos i povrće; (T2) file bez paradajz sosa; (T3) područje trbušnog operkuluma/režnja sa paradajzom i povrćem i (T4) područje trbušnog operkuluma/režnja bez paradajz sosa. Nakon mariniranja potapanjem u salamuri, stavljeni su u vakum pakete i kuvani. Mikrobiološka i približna analiza dala je rezultate u okviru standarda koji zahtevaju važeći zakoni. Senzorna analiza je pokazala stope prihvatljivosti iznad 70%. Prema senzornoj proceni i nameri kupovine, najprihvaćenija su jela pripremljena od fileta i komada isečenih iz područja trbušnog operkuluma/režnja, sa dodatkom paradajz sosa i povrća, bez razlike između delova korišćenih u pripremi jela. Zbog toga je u izradi gotovih „moqueca" jela izvodljiva upotreba manje vrednog mesa (područje trbušnog opekuluma/režnja).

Ključne reči: *„moqueca", riblji proizvod, gotova hrana, bezbednost hrane, Pseudoplatistoma reticulatum, Pseudoplatistoma corruscans.*

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Original Scientific Paper

Assessment of marketed table egg quality originating from different production systems

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A b s t r a c t: The present study evaluated the quality of marketed table eggs originating from enriched cage, barn, organic or free-range production systems. Table eggs from the free-range production system had the highest diameter, the lowest shape index and the highest frequency of normal-shaped table eggs. In addition, the lightest shell colour was found in table eggs from the free-range production system. The highest shell thickness was found in table eggs from the enriched cage production system, while the lowest shell thickness was found in table eggs from the free-range production system. Table eggs from organic and free-range production systems had better internal quality and freshness (lower albumen and yolk pH values, and a higher albumen and Haugh index) compared to table eggs from enriched cage and barn production systems. Compared to the other production systems, the best physical quality traits were recorded in table eggs from the free-range production system (the highest egg weight, weight and proportion of yolk, but the lowest weight and proportion of shell and albumen). In addition, the lightest yolk colour was found in table eggs from the organic production system. In conclusion, table eggs from organic and free-range production systems are of better overall quality compared to those from enriched cage and barn production systems.

Keywords: *albumen quality, egg freshness, eggshell colour, eggshell quality, yolk quality.*

Introduction

Owing to their highly competitive price compared to other foods, the absence of cultural and religious obstacles to their consumption, and their dietary and nutritional qualities, table eggs are consumed on a massive scale throughout the world (*Čobanović et al., 2021; Dalle Zotte et al., 2021*). A number of factors could influence the quality of table eggs within the production chain, including hen breed, genotype, physiological status and laying age, along with the production system, feeding strategy, eggshell colour, and egg processing and storage conditions (*Dalle Zotte et al., 2021; Djokić et al., 2022*).

Referring to production systems, battery cages played an important role in traditional table egg production since the 1950s (*Lordelo et al., 2017; Dalle Zotte et al., 2021; Yurtseven et al., 2021*). The objectives of this production system were to provide for laying hen's health and product safety, and minimise workload, but maximise profit and productivity (*Dalle Zotte et al., 2021*). However, there were serious hen welfare concerns regarding conventional cages, because insufficient space and restricted movement provide no or few opportunities for laying hens to express natural behaviours, such as nesting, perching, foraging and wing flapping, which led to metabolic and skeletal disorders (*Philippe et al., 2020; Yurtseven et al., 2021*). For these reasons, the breeding of laying hens in conventional cages in the European Union (EU) has been banned since 2012 and shortly afterwards in Serbia (*Terčič et al., 2012; Pavlović et al., 2020; Philippe et al., 2020; Dalle Zotte et al., 2021*).

Conventional cages were then replaced by alternative production systems, including enriched cages and non-cage systems (barn, free-range and organic), which provide more available space and specific resources including nest boxes, perches, and pecking and scratching areas (*Terčič et al., 2012; Pavlović et al., 2020; Philippe et al., 2020*). Enriched cage production systems are characterised by structural improvements aiming at enhancing hen welfare and typically consist of multiple tiers of cages installed in environmentally controlled poultry houses. Enriched cages must provide a minimum

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floor space allowance of 750 cm^2 per hen, of which 600 cm^2 is 45 cm high, a feeding area of at least 10 cm^2 for each hen and at least 10 cm for water supply, nest, littered area to express scratching and pecking behaviour, 15 cm of perch and a claw shortening device (*Lordelo et al., 2017; Pavlović et al., 2020; Dalle Zotte et al., 2021*).

In barn production systems, laying hens are reared on deep littered floors in a confined poultry house under completely controlled ambient conditions (*Lordelo et al., 2017; Dalle Zotte et al., 2021*). In this production system, laying hens are provided with automated feeding and drinking systems, perches and stepping rails to automated egg collection nest boxes (*Samiullah et al., 2017*).

In a typical free-range production system, laying hens are kept in poultry houses, which are often very similar to those in the barn production system, but birds also have access to a grassed outdoor free-range area (*Samiullah et al., 2017*). The outdoor free-range area provides a natural environment, ease of movement and enough space to allow the laying hens to express their natural behaviours in addition to exposure to sunlight, fresh air, and plenty of water and unlimited free dietary components like pastures, forages, plants, weeds, earthworms, worms and small insects (*Bughio et al., 2021; Dalle Zotte et al., 2021; Yurtseven et al., 2021*).

Organic production of table eggs, regulated by the Council Regulation (EC) 834/2007 (*European Union, 2007*), relies on a number of specific and restrictive production standards, including the provision of organic feed that must be free of synthetic additives and genetically modified organisms (*Lordelo et al., 2017; Dalle Zotte et al., 2021*).

Each production system has its own advantages and disadvantages, which is the main reason why all four production systems (*enriched* cage, barn, free-range and organic) still exist in Serbia and many other countries. The heterogeneity in production system, and consequently in management, can directly affect the table egg quality. However, scientific investigations into the effects of the production system on table egg quality are limited and have shown uncertainty, discrepancy and contradictory findings. Namely, table eggs from enriched cage production systems may contain more carotenoids and vitamins, as a result of chemical additives used in commercial feed mixtures, which are forbidden in organic production systems (*Dalle Zotte et al., 2021*). In contrast, some authors (*Samiullah et al., 2017; Yurtseven et al., 2021*) reported that table eggs from free-range production systems are preferable to those from other systems in terms of shell weight and thickness, albumen index and Haugh index. Other authors (*Dalle Zotte et al., 2021*) found that hens' diets in this production system may vary depending on type and quantity of herbs consumed and the ingestion of small invertebrates, thus leading to table eggs with different quality traits. Despite the fact that many consumers perceive organic table eggs as a higher quality food product and are, therefore, also willing to pay a higher price, a recent study reported (*Dalle Zotte et al., 2021*) that those eggs have lower contents of protein, fat and ash compared to table eggs from other production systems, indicating their lower quality. For barn production systems, the scientific literature reports their negative influence on table egg quality traits (weight, composition, strength, cleanliness, bacterial contamination and conservation of those qualities), with serious consequences on profitability (*Philippe et al., 2020*).

In spite of the role of different production systems on table egg quality, only scarce data and heterogeneous results are available on the quality traits of marketed table eggs, although such data are of paramount importance for consumers (*Lordelo et al., 2017; Dalle Zotte et al., 2021*). In fact, the problem for table egg consumers is that at the time of purchase (and even consumption), labels for marketed table eggs do not provide any information about the hen's breed, genotype, age or feed formulation, which are very important for the product quality. Therefore, the aim of this study was to determine the effects of different production systems (enriched cage, barn, free-range and organic) on the quality of marketed table eggs.

Materials and Methods

The study was conducted on 80 marketed table eggs obtained from brown egg-laying hens. Four large egg cartons of table eggs (n = 20) were purchased at the same local retail market, located in Belgrade, Serbia, each originating from a different egg production system (enriched cage, barn, free-range and organic). Table eggs from each production system were at the beginning of shelf life (fifth day after laying), class A and size M (53–63 g). All table eggs were kept on shaved ice (at 4 ± 1°C) in a cooler box and transported within one hour to the Sensory Analysis Laboratory (Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade) for further analysis.

Eggshell quality indicators

Determination of table egg weight

The weight of table eggs was determined by measuring the weight of each egg on an electronic scale (WPS 600/C, Radwag, Radom, Poland) with an accuracy of ±0.05 g. After determining the weight, table eggs were classified based on the Serbian regulation (2019): XL – very large (≥73 g); L – large (from 63 g to 73 g); M – medium (from 53 g to 63 g); and S – small (<53 g).

Determination of table eggs with cracks

Eggshells were visually inspected for cracks. The frequency of table eggs with cracks (%) was determined by calculating the number of broken eggs and dividing by the total number of tested eggs.

Determination of eggshell cleanliness

Eggshell cleanliness was examined in two ways: i) by examining for the presence of dirt on the eggshell; ii) by examining for the degree of eggshell cleanliness. Eggshell was considered clean when dirt was observed on less than 5% of the shell area (*Philippe et al., 2020*). The degree of eggshell cleanliness was determined using a five-point scale as follows (*Attia et al., 2014*): grade 5 – excellent (absence of dirt and traces of faecal material and/or bedding on eggshell); grade 4 – remarkably clean (remarkably clean and without traces of faecal material and/or bedding on eggshell); grade 3 – good (eggs have a clean shell and an acceptable appearance, with no traces of faecal material and/or bedding); grade 2 – fair (eggshell is dirty, but there are no traces of faecal material and/or bedding); grade 1 – dirty eggs (eggshell is dirty and there are faecal material and/or bedding present on the shell).

Determination of egg shape index

Egg shape index was determined by measuring the length and width of the egg in millimetres using a digital calliper (Precision Measuring, China) with an accuracy of 0.01 mm. The egg shape index was then calculated based on the following formula (*Yang et al., 2009*): Egg shape index = (Egg length / Egg width) × 100. Table eggs were classified based on the shape index as follows (*Duman et al*., 2016): i) sharp eggs − shape index less than 72; ii) normal (standard) eggs − shape index between 72 and 76; iii) round eggs − shape index greater than 76.

Determination of eggshell weight and percentage

The weight and percentage of the eggshell were determined after breaking the eggs and separating the content of the eggs (albumen and yolk) with an egg separator. Before measuring the eggshell weight, the inner membrane was not removed, and the shell was wiped with a paper towel. The shell weight was determined by measuring on an electronic scale (WPS 600/C, Radwag, Radom, Poland) with an accuracy of ± 0.05 g. After determining the eggshell weight, the eggshell percentage (%) was determined based on the following formula: Eggshell percentage = (Egg weight / Eggshell weight) × 100.

Determination of eggshell thickness

The eggshell thickness with the inner membrane was determined by measuring its thickness in millimetres on the sharp, equatorial and blunt parts of the egg using a digital calliper (Precision Measuring, China) with an accuracy of 0.01 mm. After determination of the eggshell thickness at the three points, the shell thickness uniformity was calculated based on the following formula (*Yan et al., 2014*): Eggshell thickness = (sharp end thickness + equatorial end thickness + blunt end thickness) / 3.

Determination of eggshell colour

Sensory and instrumental methods were used for determination of eggshell colour. The sensory colour of eggshell was determined by an analytical panel of three experienced sensorists based on the Grading eggshell colour standard, whereby colour scores ranged from 1 (light) to 5 (dark) (*Karabasil et al., 2020*). Instrumental eggshell colour measurements were determined on the sharp, equatorial and blunt part of the egg using a portable colorimeter (NR110, 3NH Technology Co., Ltd, Shenzhen, China) equipped with a 8 mm aperture, 2° viewing angle, and D65 illuminant. Before measurement, the colorimeter was calibrated according to the manufacturer's instructions. The average L*, a* and b* values of three measurements on each part of the egg were taken as a final result. After determination of L*, a* and b* average values, the E value on the sharp, equatorial and blunt part of the egg was calculated based on the following formula (*Baylan et al., 2017*): E value= $(L^{*2} + a^{*2} + b^{*2})^{1/2}$. Using the obtained E values on the sharp, equatorial and blunt part of the egg, the E value of the whole egg was determined based on the following formula (*Baylan et al., 2017*): $E_{\text{whole egg}}$ value = E value = $(E_{\text{Sharp end}} + E_{\text{Equatorial part}} + E_{\text{Blunt end}}) / 3$.

According to the $E_{whole\ egg}$ value, table eggs were classified as follows (*Baylan et al., 2017*): i) dark eggs ($E_{whole\ egg} < 64$); ii) medium ($E_{whole\ egg}$ value between 64 and 67); iii) light eggs ($E_{whole\ egg} > 67$).

Albumen quality indicators

Determination of albumen weight and percentage

Albumen weight was determined after breaking the eggs and separating the shell and yolk with an egg separator. Determination of albumen weight was performed by measuring on an electronic scale (WPS 600/C, Radwag, Radom, Poland) with an accuracy of ± 0.05 g. After determination of the albumen weight, the percentage of albumen (%) was determined based on the following formula: Albumen percentage = (Egg weight / albumen weight) × 100.

Determination of albumen pH

Albumen pH was determined in three different points using a pH meter (Inolab pH Level 1, WTW Gmbh Weilheim, Germany) equipped with a glass electrode (Hamilton biotrode, Bonaduz, Switzerland). The pH meter was calibrated with standard solutions pH 7.00±0.01 and pH 4.00±0.01 at 20°C (Reagecon Biomedical, Ireland) according to the manufacturer's instructions. The average of the three pH measurements was taken as the final result.

Determination of Haugh index

The determination of Haugh index was performed by measuring the egg weight and albumen thickness. Egg weight was measured as previously described. Thereafter, the eggshell was broken and egg content was transferred into a Petri dish, and then albumen height was measured using a digital calliper (Precision Measuring, China) with an accuracy of 0.01 mm. The Haugh index was determined based on the following formula (*Haugh, 1937*): Haugh index = $100_{log} \times (H + 7.51 - 1.7 \times W^{0.37})$, with W = egg weight (g) and H = albumen height (mm).

Determination of albumen index

For the determination of albumen index, the eggshell was broken and egg content was transferred into a Petri dish. Afterwards, albumen height (at a distance of 1 cm from the edge of the yolk), length (from the longest edges of the albumen) and width (from the widest edges of the albumen) were measured using a digital calliper (Precision Measuring, China) with an accuracy of 0.01 mm. The height, length and width of the albumen were determined without separating from the yolk. After determination of albumen height, length and width, the albumen index was calculated based on the following formula (*Baylan et al., 2017*): Albumen index = (Albumen height / Albumen length + Albumen width) × 100.

Yolk quality indicators

Determination of yolk weight and percentage

Yolk weight was determined after breaking the eggs and separating the shell and albumen with an egg separator. The yolk weight was measured on an electronic scale (WPS 600/C, Radwag, Radom, Poland) with an accuracy of ± 0.05 g. After determination of yolk weight, the yolk percentage (%) was determined based on the following formula: Yolk percentage = (Egg weight / Yolk weight) × 100.

Determination of yolk pH

Yolk pH value was determined in three different points using a pH meter (Inolab pH Level 1, WTW Gmbh Weilheim, Germany) equipped with a glass electrode (Hamilton biotrode, Bonaduz, Switzerland). The pH meter was calibrated with standard solutions pH 7.00±0.01 and pH 4.00±0.01 at 20°C (Reagecon Biomedical, Ireland) according to the manufacturer's instructions. The average of the three pH value measurements was taken as the final result.

Determination of yolk index

For the determination of yolk index, the eggshell was broken and egg content was transferred into a Petri dish. Yolk width and height were measured using a digital calliper (Precision Measuring, China) with an accuracy of 0.01 mm. The yolk width and height were determined without separating from albumen. After determination of yolk width and height (on its middle), the yolk index was calculated based on the following formula: Yolk index = (Yolk height / Yolk width) × 100.

Determination of yolk colour

Sensory and instrumental methods were used for determination of yolk colour. In order to determine yolk colour, the eggshell was broken and egg content was transferred into a Petri dish (50 mm in diameter). The sensory colour of yolk was determined by an analytical panel of three experienced

sensorists based on the Roche Yolk Colour Fan standard (DSM, Basel, Switzerland), whereby colour scores ranged from 1 (pale yellow) to 16 (dark orange). Instrumental yolk colour measurements were determined using a portable colorimeter (NR110, 3NH Technology Co., Ltd, Shenzhen, China) equipped with a 8 mm aperture, 2° viewing angle, and D65 illuminant. Before measurement, the colorimeter was calibrated according to the manufacturer's instructions. During instrumental measurement of yolk colour, colorimeter aperture was leaned on the vitelline membrane. The average L*, a* and b* values of three yolk colour measurements were taken as a final result.

Statistical analysis

Statistical analysis of the results was conducted with SPSS software (Version 23.0, IBM Corporation, Armonk, NY, USA) (*SPSS, 2015*). Before any formal statistical analysis, data were checked for linearity, normality of residuals (Shapiro–Wilk and Kolmogorov–Smirnov tests), outliers, and homogeneity of variance (Levene's test), and successfully passed all tests. According to the production system, marketed table eggs were divided into four groups: i) enriched cage (n = 20); ii) barn (n = 20); iii) organic (n = 20); and iv) free-range (n = 20). One-way analysis of variance (ANOVA) was performed to detect significant differences of various eggshell, albumen and yolk quality parameters between different egg production systems. Significant means at $P \leq 0.05$ were further compared using Tukey's test (multiple comparisons). All results were described by descriptive statistics − mean value and standard error of the mean. The Chi-squared test was used to determine the frequency of cracked eggs, dirty eggs and eggs with different shape index and shell colour with respect to the egg production system. In all tests, statistical significance was accepted at $P < 0.05$, tendencies were accepted at $0.05 < P < 0.10$.

Results and Discussion

Effects of production system on the external quality traits of table eggs

Effects of the production system on shell quality of table eggs are depicted in Table 1. The present investigation found the highest ($P<0.05$) egg weight in eggs from the free-range production system, while the lowest ($P<0.05$) egg weight was recorded in eggs from the organic production system. The results of previous studies on the impact of the production system on the egg weight are not consistent. Some investigations (*Djukić-Stojčić et al., 2009; Lordelo et al., 2017; Samiullah et al., 2017; Philippe et al., 2020*) found a higher egg weight in laying hens reared in the enriched cage production system compared to those from barn and free-range production systems. Other authors (*Samiullah et al., 2017*) found the largest egg weight in laying hens from the free-range production system. On the other hand, in some studies (*Mugnai et al., 2009; Rakonjac et al., 2017; Rakonjac et al., 2018*), the influence of the production system on the egg weight was not determined. Contradictory results of the production system impact on the egg weight can be attributed to a number of influencing factors such as the flock age at the time of sampling, ambient temperature, diet and breed of laying hens (*Samiullah et al., 2017*). Therefore, it is impossible to determine the exact reason responsible for the observed differences between production systems obtained in this study, because the rearing conditions on the farms were not known and controlled (except the sell-by date). There is a possibility that laying hens from free-range production system were older than laying hens from other production systems, and it is well known that the egg weight (and of each of their components) increases with increasing hen age (*Philippe et al., 2020*).

In this study, no effects of production system on the percentage of cracked eggs were found ($P>0.05$, Table 1). Contradictory results were obtained by *Lordelo et al. (2017)*, who found a higher frequency of cracked eggs from enriched cage production system. In contrast, *Patterson et al. (2001)* found a higher frequency of cracked eggs from specific production (organic and free-range) systems compared to those from conventional (enriched cage and barn) production systems.

During the examination of egg cleanliness, the percentage of dirty eggs and eggshell dirtiness scores did not differ ($P>0.05$) between production systems (Table 1). In contrast, some authors (*Englmaierova et al., 2014*) reported a higher degree of shell dirtiness and contamination in table eggs from the enriched cage production system compared to those from barn and free-range production systems, while others (*Djukić-Stojčić et al., 2009; Philippe et al., 2020*) found a higher percentage of dirty eggs in the free-range production system compared to other production systems. Eggs laid outside the nest are an important factor that negatively affects the production profitability, because they are associated with a higher degree of dirtiness and bacterial contamination,

higher frequency of being cracked, as well as higher workload as a result of manual egg collection (*Philippe et al., 2020*). The cleanliness of table eggs originating from organic and free-range production systems directly depends on the season, the number of misplaced eggs and the work organisation on the farm (frequency of egg collection and nest cleaning) (*Djukić-Stojčić et al., 2009*). Therefore, the obtained results can be explained by the fact that the study was conducted during summertime in the temperate climate zone, when sunny and dry days prevail with very rare and short-term precipitation, which resulted in a lower degree of shell dirtiness of table eggs from organic and free-range production systems.

In this investigation, the lowest (P<0.05) egg width was recorded in eggs from organic production system, while the highest (P<0.05) egg length and the lowest (P<0.05) egg shape index were found in eggs from free-range production system (Table 1). Also, the highest (P<0.05) percentage of normal-shaped eggs and the lowest (P<0.05) percentage of round eggs was recorded in the free-range production system (Table 1). Other studies have found a higher egg shape index in table eggs from the conventional cage and barn production systems than in those from the enriched cage production system (*Philippe et al., 2020*). However, some studies have not found any relationship between the production system and egg shape index (*Đukić-Stojčić et al., 2009; Rakonjac et al., 2018; Dalle Zotte et al., 2021*). Although the egg shape index may seem like a less important quality indicator of table eggs, it affects the percentage of cracked eggs, whereby the sharp egg shape increases the risk of eggs rolling out of the nest, which can result in shell damage (*Philippe et al., 2020*). Likewise, round eggs and unusually long eggs do not fit in cardboard packaging, making them more likely to be damaged or broken during handling, packaging, transportation and storage compared to normal-shaped eggs (*Čobanović et al., 2021*). Accordingly, it can be argued that table eggs from free-range production system have the most acceptable shape and the lowest risk of breakage throughout the table egg supply chain.

In this study, the lightest (P<0.05) eggshell colour (the lowest eggshell sensory colour scores and the highest $E_{whole\ egg}$ value) and highest (P<0.05) percentage of light eggs were found in eggs from the free-range production system (Table 1). Contrary to the results obtained in this study, *Djukić-Stojčić et al. (2009)* did not find any impact of the production system on the eggshell colour, while *Lordelo et al. (2017)* determined a darker shell colour in eggs originating from the enriched cage production system. Although shell colour is not an indicator of nutritional composition and/or internal quality of table eggs, most consumers show a greater tendency to purchase brown eggs, paying special attention to the intensity and uniformity of shell colour within the cardboard packaging (*Lordelo et al., 2017*). It is unlikely that the difference in eggshell colour is a consequence of the production system impact, but can be ascribed to the different breed, age or physiological state of the laying hens (*Lordelo et al., 2017*). However, it is very difficult to determine the exact reasons responsible for the observed differences between production systems obtained in this study, because the rearing conditions on the farms were not known or controlled (except the sell-by date). As mentioned before, there is a possibility that laying hens from the free-range production system were older than laying hens from other production systems, which may explain the lighter shell colour of table eggs from this production system. This phenomenon is directly related to the increase in the egg size and weight as hens age, which occurs without any proportional increase in the amount of protoporphyrin pigments deposited on the eggshell surface. As a consequence, the larger eggshell surface of older laying hens is covered with an unchanged amount of pigments, which results in a lighter egg colour (*Lordelo et al., 2017; Samiullah et al., 2017; Čobanović et al., 2021*).

The weight, percentage and thickness of the eggshell, together with the egg shape index, are important physical indicators of the table egg quality, considering that they affect the resistance of the shell to breakage and, consequently, reduce the percentage of cracked eggs during handling, packaging, transportation and storage (*Dalle Zotte et al., 2021*). Although each production system for laying hens has a number of differences that can have a significant impact on the eggshell characteristics, literature data indicate that the production system is not a decisive factor influencing the formation of eggshell properties (*Lordelo et al., 2017; Samiullah et al., 2017; Dalle Zotte et al., 2021*). Factors that are well known to influence the eggshell characteristics are the age of the laying hens, diet, stress and light regime (*Dalle Zotte et al., 2021*). Contrary to the results of previous studies, in the present study, the lowest (P<0.05) eggshell weight, eggshell percentage and eggshell thickness were determined in eggs originating from the free-range production system (Table 1), which is in accordance with the results obtained by *Terčič et al. (2012)* and *Dalle*

Table 1. Effects of production system on shell quality of table eggs (n=80).

Production system	Enriched cage	Barn	Organic	Free-range	*P* - value	Significance
N	20	20	20	20		
Egg weight (g)	55.52 ± 0.47^{a}	55.85 ± 0,39^{a}	53.54 ± 0.63^{b}	59.08 ± 0.71^{c}	<0.0001	*
Cracked eggs (%)	5.00	5.00	0.00	0.00	0.5678	ns
Dirty eggs (%)	10.00	0.00	0.00	5.00	0.2828	ns
Eggshell dirtiness scores	1.55 ± 0.21	1.45 ± 0.14	1.25 ± 0.10	1.55 ± 0.18	0.5264	ns
Egg width (mm)	43.58 ± 0.17^{a}	43.57 ± 0.13^{a}	42.71 ± 0.16^{b}	43.65 ± 0.20^{a}	0.0002	*
Egg length (mm)	55.72 ± 0,31^{a}	55.89 ± 0.31^{a}	54.85 ± 0.37^{a}	58.71 ± 0.40^{b}	<0.0001	*
Egg shape index	78.26 ± 0.52^{a}	78.00 ± 0.52^{a}	78.05 ± 0.52^{a}	74.42 ± 0.60^{b}	<0.0001	*
Egg shape quality classes						
Sharp eggs (%)	0.00	0.00	0.00	20.00	0.0055	*
Normal eggs (%)	25.00^{a}	15.00^{a}	15.00^{a}	55.00^{b}	0.0130	*
Round eggs (%)	75.00^{a}	85.0^{0}a	85.00^{a}	25.00^{b}	<0.0001	*
Eggshell colour (sensory)	3.34 ± 0.13^{a}	3.30 ± 0.09^{a}	3.25 ± 0.12^{a}	1.93 ± 0.08^{b}	<0.0001	*
$E_{whole\ egg}$	66.63 ± 0.65^{ab}	65.2 ± 0.61^{a}	68.38 ± 0.60^{b}	79.74 ± 0.76^{c}	<0.0001	*
Egg colour quality classes						
Light eggs (%)	55.00^{a}	15.00^{b}	60.00^{a}	100.00^{c}	<0.0001	*
Normal (medium) eggs (%)	25.00^{a}	65.00^{b}	40.00^{ab}	0.00^{c}	0.0001	*
Dark eggs (%)	20.00	20.00	0.00	0.00	0.0308	*
Eggshell weight (g)	7.24 ± 0.14^{a}	7.48 ± 0.10^{a}	7.21 ± 0.14^{a}	6.71 ± 0.14^{b}	0.0008	*
Eggshell percentage (%)	13.01 ± 0.22^{a}	13.41 ± 0.19^{a}	13.49 ± 0.29^{a}	11.39 ± 0.28^{b}	<0.0001	*
Eggshell thickness (mm)	0.39 ± 0.01^{a}	0.30 ± 0.01^{b}	0.22 ± 0.01^{c}	0.14 ± 0.004^{d}	<0.0001	*

Legend: Level of significance: * $P < 0.05$; ns: not significant ($P > 0.05$); different letters in the same row indicate a significant difference at $P < 0.05^{(a–d)}$.

Zotte et al. (2021). On the other hand, the highest (P<0.05) shell thickness was recorded in eggs originating from the enriched cage production system (Table 1). This can be attributed to a better realisation of the genetic potential of the laying hens from the enriched cage production system due to better ambient environment and optimised nutrition, which might have led to more efficient utilisation of calcium and phosphorus during the process of eggshell formation (*Philippe et al., 2020; Bughio et al., 2021*). In barn, organic and free-range production systems, laying hens have a larger available floor surface, which significantly stimulates the movement of the animals, so minerals are used more for development and preservation of bones than for formation of eggshell (*Philippe et al., 2020*).

Effects of production system on the internal quality traits of table eggs

Effects of production system on internal quality of table eggs are depicted in Table 2. The present investigation found the lowest (P<0.05) percentages of albumen, but the highest (P<0.05) weight and percentage of yolk in eggs originating from the free-range production system. The ratio of table egg components is of little importance for consumers, but, on the other hand, it is of great importance for the egg product industry, because egg yolk has a higher market value than does albumen (*Lordelo et al., 2017*). There are contradictory results in the literature on the impact of the production system on physical internal quality traits of table egg quality. In some cases, it was found

that table eggs originating from free-range/organic production systems have a higher proportion of albumen and lower proportion of yolk compared to table eggs from conventional production systems (*Lordelo et al., 2017; Samiullah et al., 2017; Dalle Zotte et al., 2021*), while in other cases, no influence of the production system on the internal quality traits of table eggs was determined (*Türker and Alkan, 2019*). The results of this study indicate that the physical internal quality indicators of table egg (weight and percentage albumen and yolk) differ in relation to the production system, confirming the great diversity of production in terms of farm conditions, genotype, diet and environmental conditions, which directly affects the quality of table eggs (*Dalle Zotte et al., 2021*). The albumen/yolk ratio in eggs is influenced by a number of factors, such as egg weight, laying hen age and genetic factors (*Dalle Zotte et al., 2021*). In this study, all table eggs were of the same weight class (size M; 53–63 g), which limited the influence of egg weight as a key factor in the proportion of albumen and yolk in the egg. Also, genetic factors cannot be considered decisive, considering that the same laying hen hybrids are generally reared in both alternative and conventional production systems (*Dalle Zotte et al., 2021*). Therefore, the identified differences could be attributed to other factors such as laying hen age, diet and welfare conditions on the farm of origin (*Philippe et al., 2020*). Accordingly, better welfare conditions in terms of greater available floor space and access to grass pasture probably contributed to increased movement of laying hens from free-range production system and probably resulted in better utilisation of nutrients and improvement of health status (especially in the birds' ovaries), which ultimately resulted in improved internal egg quality.

Although the table eggs tested in this study were of the same age and originated from the same local retail market where they were stored in refrigerated display cabinets at a temperature of +7±1°C, table eggs from enriched cage and barn production systems had a higher (P<0.05) width and length of albumen and lower (P<0.05) albumen index and higher pH of albumen and yolk compared to those from organic and free-range production systems (Table 2). In addition, the highest (P<0.05) albumen height, as well as the highest (P<0.05) albumen and Haugh index were recorded for organic eggs. Similar results were obtained in earlier studies (*Djukić-Stojčić et al., 2009; Lordelo et al., 2017; Samiullah et al., 2017; Rakonjac et al., 2018*). The albumen pH of a newly laid eggs is between 7.6 and 7.9 (*Philippe et al., 2020; Dalle Zotte et al., 2021*), while the pH of fresh eggs ranges from 7.6 to 8.5 (*Abdel-Nour et al., 2011; Eke et al., 2013; Čobanović et al., 2021*). During storage, albumen protein is decomposed and water and carbon dioxide are progressively lost from the egg, leading to a decrease in albumen height and an increase in albumen and yolk pH, resulting in a lower albumen, yolk and Haugh index (*Lordelo et al., 2017; Philippe et al., 2020; Dalle Zotte et al., 2021*). Alterations in the albumen pH are very intense during the first four days of storage, when it reaches values around 9, while after eight days the pH reaches values around 9.15 (*Dalle Zotte et al., 2021*). This indicates that albumen pH values of table eggs from organic and free-range production systems were characteristic for eggs stored for about four days, while eggs from enriched cage and floor production systems had pH values typical for eggs stored for about eight days (*Dalle Zotte et al., 2021*). Accordingly, the results of this study suggest that table eggs from enriched cage and barn production systems have a greater ability to support bacterial growth and are not suitable for longer storage durations. The better internal quality and freshness of table eggs from organic and free-range production systems can be explained by lower exposure of laying hens to stress and ammonia from the bedding, and higher vitamin C content in albumen due to intake of fresh grass as a result of access to pasture, which lowers pH of albumen and yolk and positively affects their consistency (*Rakonjac et al., 2018; Philippe et al., 2020; Dalle Zotte et al., 2021*).

The present study found the lightest (P<0.05) yolk colour (highest L* value, but the lowest a* value and sensory colour scores) in table eggs originating from the organic production system, which is in line with previous investigations (*Terčić et al., 2012; Lordelo et al., 2017; Dalle Zotte et al., 2021*). The intensity of yolk colour primarily depends on the content of carotenoid pigments in laying hen diet, with darker yellow yolk colour being more desirable for consumers in many countries (*Samiullah et al., 2017; Philippe et al., 2020*). In conventional poultry farming, synthetic pigments, xanthophylls, are commonly used in commercial feed mixture for laying hens to obtain a darker yellow yolk colour. In contrast, the use of synthetic xanthophyll pigments in laying hen diets in organic production systems is banned in the EU (*Lordelo et al., 2017; Samiullah et al., 2017; Rakonjac et al., 2018*). This may explain why table eggs from the *enriched* cage and barn production systems have more intense yellow yolk colour compared to eggs from organic production system (Table 2). However, the results of other studies (*Lordelo et al., 2017; Rakonjac et al., 2018*) found that the access of

laying hens to the pasture, as in the case of organic and free-range production systems, allows birds to eat fresh grass, which is a rich source of carotenoid pigments and results in an increase in the yolk colour intensity. Some authors (*Hammershøj and Johansen, 2016*) found that the access of laying hens to the grass pasture in the organic production system can contribute to a two-fold increase of carotenoid pigments in table eggs. However, in free and organic production system, the availability, quality, quantity and type of grass, as well as time spent on the pasture are very important factors that affect the table egg quality and can vary significantly in different geographical areas and during different seasons (*Hammershøj and Johansen, 2016; Lordelo et al., 2017; Rakonjac et al., 2018*). Due to the previously mentioned reasons and the fact that laying hens from the enriched cage and barn production systems consume the same amount of synthetic carotenoids throughout the year, it is generally considered that egg yolks from free-range and organic production systems are of lighter yellow colour (*Dalle Zotte et al., 2021*). Hence, the results of this study are in accordance with the usual observations that table eggs from enriched cage and barn production systems have a darker yellow yolk colour.

In addition, a lighter (P<0.05) yolk colour was recorded in table eggs from the free-range production system compared to in those from enriched cage and barn production systems (Table 2), which is agreement with the results obtained by *Djukić-Stojčić et al. (2009)*. This indicate that the amount of xanthophyll pigments in the diet for laying hens from free-range production system was lower than in those from conventional production systems. This can be ascribed

Table 2. Effects of production system on albumen and yolk quality of table eggs (n=80).

Production system	Enriched cage	Barn	Organic	Free-range	*P* - value	Significance
N	20	20	20	20		
Albumen quality parameters						
Albumen weight (g)	35.50 ± 1.67	37.24 ± 1.42^{a}	31.93 ± 0.92^{b}	33.05 ± 0.63	0.0135	*
Albumen percentage (%)	59.05 ± 1.05^{a}	60.17 ± 0.69^{a}	58.26 ± 0.82^{a}	55.88 ± 0.59^{b}	0.0027	*
Albumen pH value	9.10 ± 0.05^{a}	9.18 ± 0.05^{a}	8.79 ± 0.05^{b}	8.82 ± 0.07^{b}	<0.0001	*
Albumen width (mm)	118.40 ± 3.92^{a}	125.10 ± 4.62^{a}	73.14 ± 3.39^{b}	75.75 ± 1.58^{b}	<0.0001	*
Albumen length (mm)	146.20 ± 1.97^{a}	146.10 ± 2.96^{a}	93.58 ± 4.02^{b}	94.88 ± 1.57^{b}	<0.0001	*
Albumen height (mm)	9.19 ± 0.80^{a}	12.59 ± 0.50^{b}	15.08 ± 1.14^{c}	12.63 ± 0.54^{b}	<0.0001	*
Haugh index	92.68 ± 4.62^{a}	106.20 ± 2.82^{b}	115.60 ± 2.95^{c}	111.10 ± 1.72^{d}	<0.0001	*
Albumen index	3.47 ± 0.29^{a}	4.75 ± 0.25^{a}	9.28 ± 0.72^{b}	7.44 ± 0.34^{c}	<0.0001	*
Yolk quality parameters						
Yolk weight (g)	15.46 ± 0.57^{a}	14.84 ± 0.39^{a}	15.16 ± 0.34^{a}	19.32 ± 0.35^{b}	<0.0001	*
Yolk percentage (%)	27.98 ± 1.01^{a}	26.54 ± 0.66^{a}	28.25 ± 0.71^{a}	32.73 ± 0.52^{b}	<0.0001	*
Yolk pH value	6.96 ± 0.10^{a}	7.13 ± 0.13^{a}	6.28 ± 0.03^{b}	6.28 ± 0.02^{b}	<0.0001	*
Yolk width (mm)	42.09 ± 0.69	42.77 ± 0.62	41.46 ± 0.63^{a}	43.94 ± 0.44^{b}	0.0237	*
Yolk height (mm)	20.41 ± 0.97	22.63 ± 0.87	22.03 ± 1.31	21.86 ± 0.55	0.4515	ns
Yolk index	49.03 ± 2.70	53.54 ± 2.36	52.71 ± 3.13	49.73 ± 1.07	0.4940	ns
Yolk colour (sensory)	13.18 ± 0.15^{a}	12.74 ± 0.25^{a}	7.67 ± 0.63^{b}	10.45 ± 0.23^{c}	<0.0001	*
L* value	43.43 ± 0.56^{a}	45.23 ± 0.58^{a}	48.60 ± 0.85^{b}	46.16 ± 0.84^{a}	0.0001	*
a* value	9.58 ± 0.22^{a}	9.63 ± 0.35^{a}	3.88 ± 0.53^{b}	5.53 ± 0.28^{c}	<0.0001	*
b* value	28.82 ± 1.19	32.47 ± 1.19	32.30 ± 1.42	30.56 ± 0.93	0.1482	ns

Legend: Level of significance: * P < 0.05; ns: not significant (P > 0.05); different letters in the same row indicate a significant difference at P < 0.05$^{(a–d)}$.

to the fact that free-range laying hens had access to the grass pasture, so they consumed less commercial feed mixture supplemented with the synthetic pigment, xanthophyll, which resulted in lighter yellow yolk colour (*Samiullah et al., 2017*).

Conclusion

This study showed that for external quality traits of table eggs, the free-range production system outperforms in egg weight and shape index, while the enriched cage production system is superior in eggshell thickness. The free-range and organic production systems are preferable for most of examined albumen quality traits. Therefore, these two production systems are better for desired quality parameters such as albumen pH value, albumen width, albumen length, albumen height, Haugh index and albumen index. In addition, the free-range production system is superior in all the yolk quality traits excluding yolk colour. It can, therefore, be concluded that table eggs from organic and free-range production systems are of better overall quality compared to those from enriched cage and barn production systems.

Ispitivanje kvaliteta konzumnih jaja poreklom iz različitih proizvodnih sistema

Nikola Čobanović, Nadja Todorović, Marija Kovandžić, Ivan Vićić, Branko Suvajdžić, Nevena Grković, Nedjeljko Karabasil

A p s t r a k t: Cilj istraživanja ovog rada bio je da se ispita kvalitet konzumnih jaja poreklom iz kaveznog, podnog, organskog i slobodnog proizvodnog sistema. Konzumna jaja iz slobodnog uzgoja imala su najveću dužinu, najmanji indeks oblika i najveću učestalost jaja normalnog oblika. Osim toga, najsvetlija boja ljuske utvrđena je kod konzumnih jaja iz slobodnog uzgoja. Najveća debljina ljuske je utvrđena kod konzumnih jaja iz kaveznog uzgoja, dok su najmanju debljinu ljuske imala konzumna jaja iz slobodnog uzgoja. Konzumna jaja iz organskog i slobodnog uzgoja imala su bolji unutrašnji kvalitet i svežinu (manja pH vrednost belanca i žumanca, a veći indeks belanca) u poređenju sa onim iz kaveznog i podnog uzgoja. U poređenju sa ostalim proizvodnim sistemima, najbolje fizičke karakteristike su utvrđene kod konzumnih jaja iz slobodnog uzgoja (najveća masa jaja i masa i procenat žumanca, a najmanja masa i procenat ljuske i masa belanca). Pored toga, najsvetlija boja žumanca je utvrđena kod konzumnih jaja iz organskog uzgoja. Na osnovu rezultata ovog istraživanja može da se zaključi da su konzumna jaja iz organskog i slobodnog uzgoja boljeg kvaliteta u odnosu na ona iz kaveznog i podnog uzgoja.

Ključne reči: *boja ljuske, boja žumanca, kvalitet belanca, kvalitet ljuske, kvalitet žumanca, svežina jaja.*

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Note: Laws should be cited normally in the text and put in the ref list. Cite them like this: The European Union (EU) food information regulation.(EU, 2011). In Serbia, the relevant laws concern labelling and advertising of food (Official Gazette of RS, 2017; 2018; 2020) and diet foods (Official Gazettel of RS…)

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