Toshihide Nishimura · Motonaka Kuroda
Editors

Koku in Food Science and Physiology

Recent Research on a Key Concept in Palatability

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Preface

In Japan, there are lots of foods on whose labels we can see the expression "*Koku* attributes." Why are "*Koku* attributes" used for lots of foods? One reason is that Japanese consumer image palatability of foods from the display of "*Koku* attributes." Japanese use the expression *Koku* in eating some foods. What kind of foods gives us the sensation of *Koku* in Japan? When they eat curry, stew, ramen noodles, cheese, and so on, the expression *Koku* is in general used. On the other hand, when they eat watermelon, Japanese pear, lemon juice, and Japanese apricot, they never use the expression *Koku*. What is different between both groups? The foods with *Koku* attributes give us the sensations such as complexity, mouthfulness, and lingeringness when we put them into our mouth and chew, while other foods without *Koku* attributes do not. These attributes are recognized when they eat foreign foods such as French foods and Chinese foods as well as Japanese foods. However, in these many foods, it is still unclear what the sensation of *Koku* is.

Several years ago, we organized "*Koku* Attribute Association" and started discussing about the definition of "*Koku* attribute." At first, we proposed that the sensation of "*Koku* attributes" is caused by lots of stimulations of taste, aroma, and texture. Furthermore, we made objective elements such as complexity, mouthfulness and lingeringness in the factor "*Koku* attributes," as five basic tastes make up taste.

Complexity is essential for foods with "*Koku* attributes," because lots of stimulations of taste, aroma, and texture are given to us in these foods and give the characteristic of the foods with "*Koku* attributes." These lots of stimulations are produced during postmortem aging, heating, and fermentation in the production of foods. Mouthfulness and lingeringness are brought by umami compounds and lipids. In the presence of umami compounds and/or lipids, many stimulations spread into oral cavity and linger in our mouth. The compounds such as *kokumi* substances, aroma compounds, and maillard peptides have already been shown to enhance mouthfulness in *Koku* attribute of foods. We think that three elements in *Koku* attributes are different in the appropriate intensity depending on foods. For example, there are the expressions such as weak, medium, and strong. Depending on the amount of umami compounds, lipids and *kokumi* compounds and so on, the *Koku* intensity should be able to be changed from weak to strong. And *Koku* attributes are possible to be objectively measured.

However, the research on *Koku* attributes have just started. Having this in mind, in this book, we asked to write manuscript of chapters to authors who are doing research on *Koku* attributes. Chapter 1 provides the definition of "*Koku* attributes." *Koku* is the unique sensation caused by lots of stimulations of taste, aroma, and texture and is constituted by three elements, that is, complexity, mouthfulness, and lingeringness. Furthermore, the compounds responsible for *Koku* enhancer are also introduced. It will help the readers to understand what "*Koku* attribute is." Chapters 2 and 3 provide accurate functions of glutamate in terms of its taste and its flavor-enhancing action. Umami compound is also shown to be important in the *Koku* attribute by enhancing continuity and mouthfulness of the overall taste experience. Chapters 4 and 5 provide compounds responsible for the formation and the enhancement of *Koku* attributes in pork sausage and beer. Umami compound and fats enhance mouthfulness and lingeringness of *Koku* attributes in pork sausages. Aroma compounds are contributing to the complexity or thickness of *Koku* attribute in beer aroma. Chapter 6 provides odor compounds which enhance *Koku* attribute of foods. They are also shown to be a phthalide compound in celery, (4Z, 7Z)-trideca-4,7-dienal in dried bonito, and rotundone in several fruits. Chapters 7, 8 and 9 provide the *kokumi* substances and their functions on the sensory characteristics of foods and their mechanisms for the enhancement of *Koku* attributes from physiology aspect. γ-glutamyl-valyl-glycine and glutathione are shown to be *kokumi* peptides and enhance oiliness, thick flavor, and aftertaste of foods. These peptides are clarified to be an agonist of calcium-sensing receptor (CaSR) and stimulate the mouse trigeminal neurons. Chapter 10 provides the overview of the study on *Koku*.

I hope that the information provided in this book shall give an outlook of the definition of "*Koku* attributes," the compounds involved in *Koku* attributes of some foods, and the physiology concerning with the sensation of "*Koku* attributes" to the novices in the field, as well as useful information for more experienced readers, and can be a concise starting book for all interested in food science and physiology of "*Koku* attributes."

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Contents

Chapter 1
Definition of "*Koku*" Involved in Food Palatability

Toshihide Nishimura

Abstract It is well known that many factors—taste, aroma, tenderness, juiciness, viscosity, and so on—are involved in food palatability. In Japan, the term "*koku*" (referring to *koku* attributes) is used in eating and evaluating to be palatable foods such as curry, stew, ramen noodles, natural cheese, and so on. Thus, it has been thought to be one of the important factors enhancing food palatability. However, *koku* has not yet been defined, and there have been few research studies on the compounds that enhance *koku* (*koku* attributes).

This chapter introduces definitions of *koku* and *koku* attributes. *Koku* is the unique sensation caused by lots of stimulation in terms of taste, aroma, and texture, which is expressed as complexity in *koku*. This is the essence of *koku* attributes. Furthermore, when this sensation is spread out without changing its characteristic flavor in our whole oral cavity, and it lingers after swallowing, we can sense strong *koku* attributes such as mouthfulness and lingeringness. Therefore, *koku* is constituted by three elements: complexity, mouthfulness, and lingeringness (continuity). Complexity in the *koku* attributes of foods is produced by aging, fermentation, and heating, causing production of various compounds. Mouthfulness and lingeringness (continuity) have recently been explained as being caused by umami compounds and/or phytosterols in onions.

Recent sensory evaluation analyses have shown that the addition of umami compounds enhances the retronasal aroma sensation, resulting in enhancement of the flavor of foods (mouthfulness). Umami compounds have also been shown to cause an effect of lingeringness of the taste sensation. Furthermore, phytosterols in onion have been confirmed to contribute to a sensation of lingeringness of the aroma—a *koku* attribute. Headspace gas chromatography analyses have shown that β-sitosterol binds with methyl propyl disulfide and hexanal. The addition of 0.02% β-sitosterol to consommé soup enhances the mouthfulness and lingeringness of the aroma sen-

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sation. On the basis of these findings, umami compounds and phytosterols are thought to be candidates for enhancement of *koku* attributes in palatable foods.

Keywords *Koku* · Complexity · Mouthfulness · Lingeringness · Umami compounds · Lipids

1.1 *Koku* Attributes in Foods

For a long time, in Japan, the term "*koku*" (referring to *koku* attributes) has been used to describe palatable foods, such as when we eat curry and, stew, ramen noodles, natural cheese, and so on. Recently, the term "*koku*" has begun appearing on the packaging of many foods such as mayonnaise, coffee, cocoa, yogurt, pudding, *kimuchi*, beer, and soy sauce. Since they are accustomed to saying that there are *koku* attributes in the foods they find palatable, the use of the term "*koku*" on food packaging makes many Japanese people think of palatable foods (Fig. 1.1). However, we recognize that "*koku*" is not necessarily a synonym for the terms "palatability" and "deliciousness." Although pear, watermelon, lemonade, and umeboshi (pickled ume) are considered palatable foods by many Japanese people, they never say that there are *koku* attributes in these foods (Fig. 1.2). These foods are not heated, conditioned (i.e., aged), or fermented, so there is a simple sensation but no sensations of complexity, mouthfulness, and lingeringness. On the other hand, foods such as curry, stew, ramen noodles, and natural cheese—which are heated, conditioned, or fermented—give us sensations such as complexity, mouthfulness, and lingeringness, and when people find the complexity, mouthfulness, and lingeringness of these foods suitable, they consider these foods palatable with *koku* attributes.

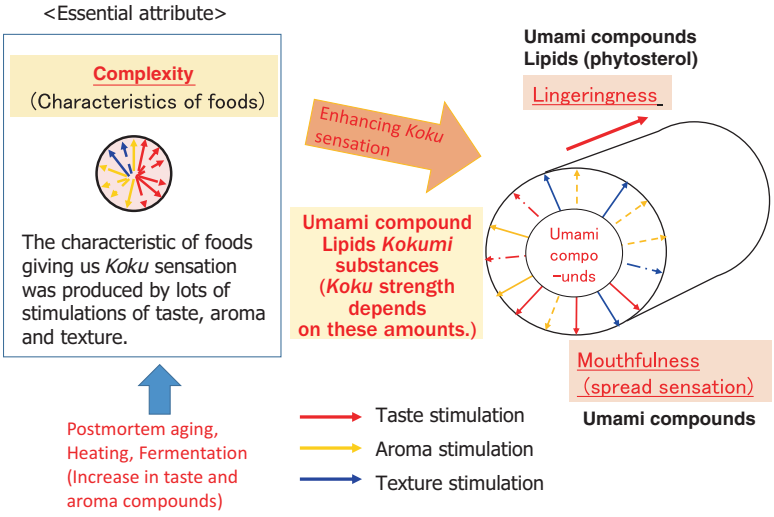


Fig. 1.1 The definition of *koku*

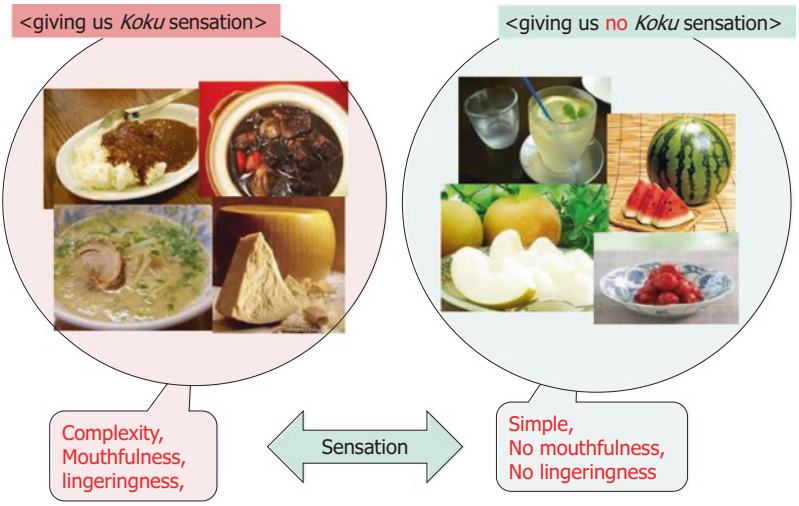


Fig. 1.2 What kind of foods convey *koku*?

However, when people find these characteristics unsuitable in a food, they consider them unpalatable because they contain too much or too little complexity, mouthfulness, and/or lingeringness. Therefore, the term "*koku*" is not a synonym for the terms "palatability" and "deliciousness." It is also thought that palatability and deliciousness are determined by subjective evaluation, whereas *koku* attributes are determined by objective evaluation. This is the first time that this concept has been proposed, because *koku* attributes have not yet been defined, and there have been few research studies on the compounds that enhance *koku* (*koku* attributes).

1.2 Definitions of *Koku* and *Koku* Attributes

1.2.1 Factors Involved in Food Palatability

Many factors—such as taste, aroma, texture (tenderness, viscosity, smoothness, juiciness, and so on), color, temperature, and shape—are involved in food palatability (Fig. 1.3). Among many factors, in general, taste is caused by taste compounds in foods, which are water soluble. Aroma is caused by aroma compounds, which are volatile compounds released from foods. Aroma is classified into orthonasal and retronasal aromas, and the latter aroma is sensed after foods are put into the oral cavity. This retronasal aroma is thought to be the most important factor involved in food palatability. Recently, an interaction between taste and retronasal aroma has been described. Previously, *koku* attributes were not classified into objective factors involved in food palatability, because the meaning of *koku* (*koku* attributes) was thought to be the same as palatability. However, it is now thought that *koku* is one of

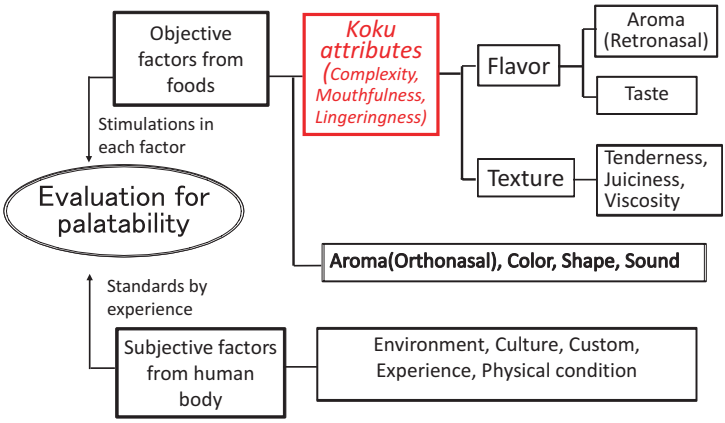


Fig. 1.3 Factors involved in food palatability

the objective factors involved in food palatability, and the sensations of complexity, mouthfulness, and lingeringness in the *koku* attributes of foods such as curry, stew, ramen noodles, and natural cheese are thought to be caused by the entirety of factors such as taste, aroma, and texture.

1.2.2 Definition of Koku *Attributes*

Given the background mentioned above, foods such as curry, stew, ramen noodles, and natural cheese—which are heated, conditioned, or fermented for a long time—possess *koku* attributes such as complexity, mouthfulness, and lingeringness (Fig. 1.1). It has been confirmed that *koku* attributes are mainly conveyed by a lot of stimulation from taste compounds, aroma compounds, and texture. Many consumers believe that palatability is caused only by the taste of a food after it is put into their oral cavity. However, when we eat a food while pinching our nose, we cannot identify what kind of food it is and do not evaluate that food as being palatable. This means that retronasal aromas play an important role in our evaluation of the palatability of foods. We also have a similar experience of finding the foods we eat unpalatable when we have a blocked nose because of catching a cold. This is the reason why we cannot sense *koku* attributes and the characteristic aromas of foods. Therefore, the number and the intensity of stimulations from taste and aroma compounds, and from texture, convey the intensity of the complexity of *koku* attributes.

There is an objective intensity in the *koku* attributes of complexity, mouthfulness, and lingeringness, and this intensity depends on the amount of stimulation from taste compounds, aroma compounds, and the compounds involved in texture. The compounds that contribute to *koku* attributes and the intensity of *koku* attributes differ depending on the food. Research on these compounds and the intensity of *koku* attributes in each food is needed.

1.3 Elements and Compounds Involved in *Koku* Attributes

When we eat foods with *koku* attributes, we sense their complexity, mouthfulness, and lingeringness. So, I propose that these three sensations are basic elements of *koku* attributes, just as the five basic elements of taste are sweetness, saltiness, sourness, bitterness, and umami (Fig. 1.4).

1.3.1 Complexity

The complexity of foods with *koku* attributes is harmoniously formed by lots of stimulation from taste compounds, aroma compounds, and texture (Fig. 1.5). When we get lots of stimulation unharmoniously from foods, we do not sense complexity. Therefore, harmony already exists in complexity and is not a basic element of *koku* attributes.

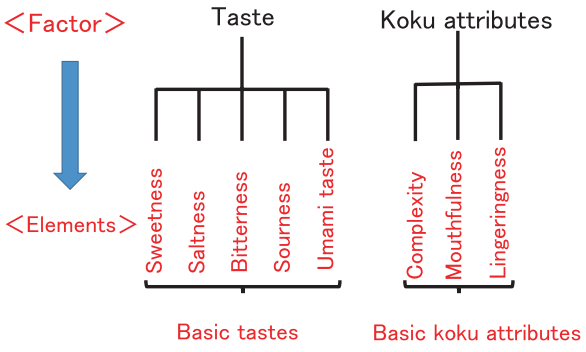


Fig. 1.4 Elements of taste and *koku* attributes

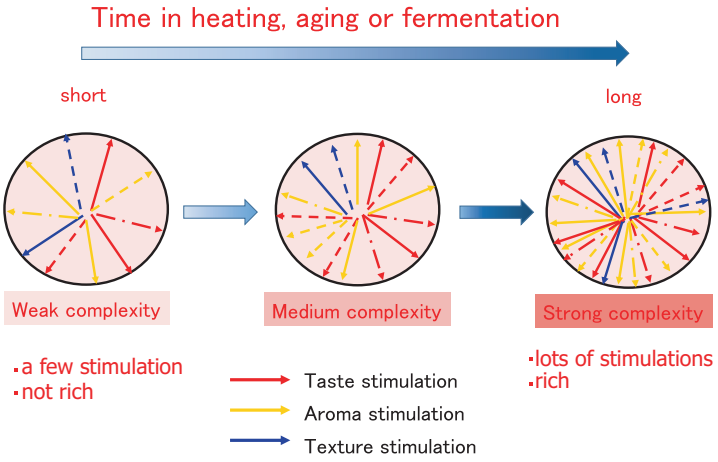


Fig. 1.5 Formation of complexity in *koku* attributes of foods

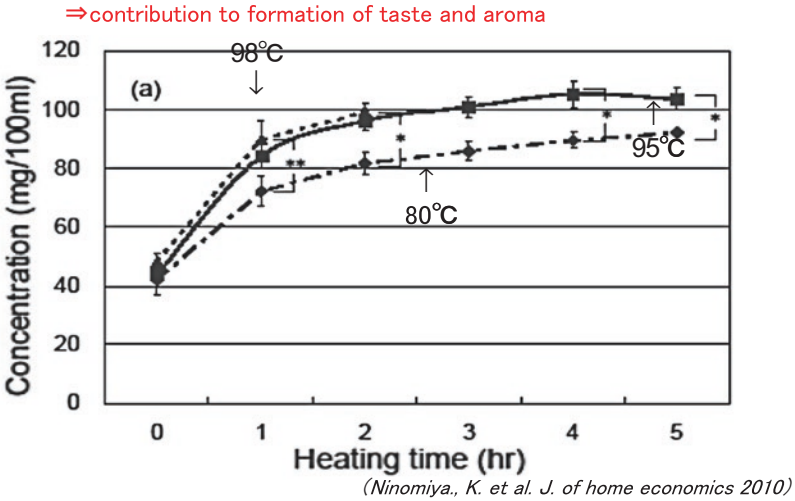


Fig. 1.6 Changes in free amino acids extracted from materials during heating

The stimulation in foods with *koku* attributes is produced by heating, fermentation, and conditioning for a long time. Stew and curry are cooked by heating for a long time. During heating, various taste compounds are extracted from foodstuffs and various aroma compounds are produced by Maillard reactions between amino acids and sugars (Fig. 1.6). Miso paste and soy sauce are produced by fermentation with microorganisms such as *Aspergillus* and lactic acid bacteria. These microorganisms produce free amino acids and peptides by protein degradation and aroma compounds by enzyme reactions and by Maillard reactions (Fig. 1.7). Natural cheeses are also conditioned for long time. During conditioning of natural cheeses, taste compounds such as free amino acids and peptides are produced through protein degradation by proteases in microorganisms. Many kinds of aroma compounds are also produced from lipids by microorganisms. In particular, the types of aroma compounds differ between natural cheeses because of the difference in the microorganisms in those cheeses.

The constituents of taste and aroma compounds involved in complexity lead to the characteristics of these foods. The intensity of the complexity in these foods can be varied by changing the duration of heating, conditioning, or fermentation, leading to differences in the flavor characteristics of these foods. We prepared a synthetic chicken extract using compounds that had been listed as constituents of the taste of chicken in a previous paper. When we omitted one free amino acid, the intensity of the retronasal aroma was found to decrease.

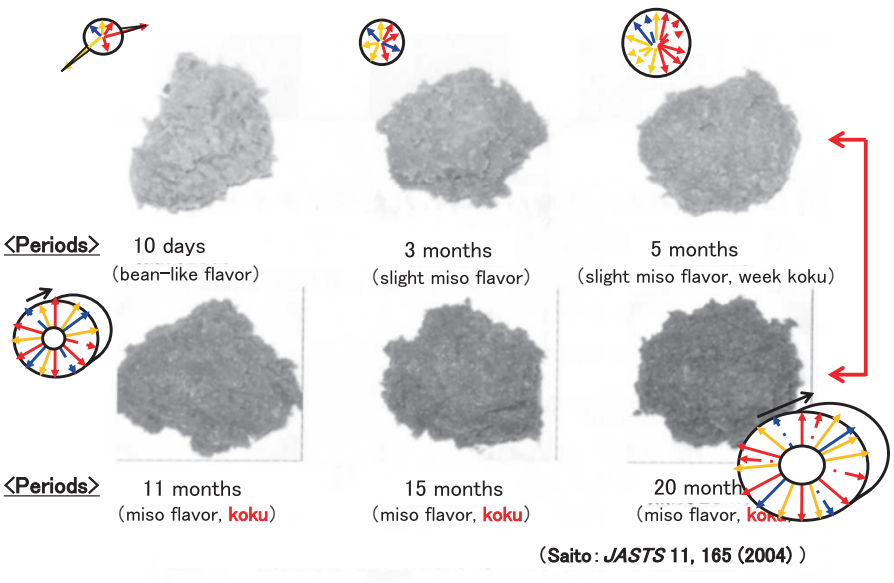


Fig. 1.7 Increase in the intensity of *koku* attributes during fermentation of miso paste

1.3.2 Mouthfulness

The mouthfulness in the *koku* attributes of foods such as curry and stew is sensed by the spread of the flavor sensation in the space of our oral cavity without changing its characteristic flavor. This spread of the flavor sensation is thought to be caused by umami substances. A miso soup concentration of 50 g/L without seasonings provides a very weak sensation in the space of our oral cavity, although it has the characteristic flavor and complexity of miso soup. The addition of umami substances to this miso soup can increase the intensity of the spread of the flavor sensation in our oral cavity without changing its characteristic flavor. Yamaguchi and Kimizuka (1979) reported that umami substances had the effect of flavor enhancers when they were added to foods. Recently, Nishimura et al. (2016a) examined the mechanism of flavor enhancement with addition of umami substances to foods. Their analysis, using a model chicken extract, clarified that addition of monosodium glutamate (MSG) to the extract increased the sensation of the retronasal aroma by 2.5 times (Fig. 1.8). This effect increased when the concentration of added MSG was increased up to a value of 0.3%. However, addition of MSG in a concentration over 0.3% made the sensation of the retronasal aroma of the extract weaker, while the intensity of the umami taste became stronger. Therefore, umami substances have the strong effect of conveying the mouthfulness *koku* attribute by enhancing flavor, especially the retronasal aroma.

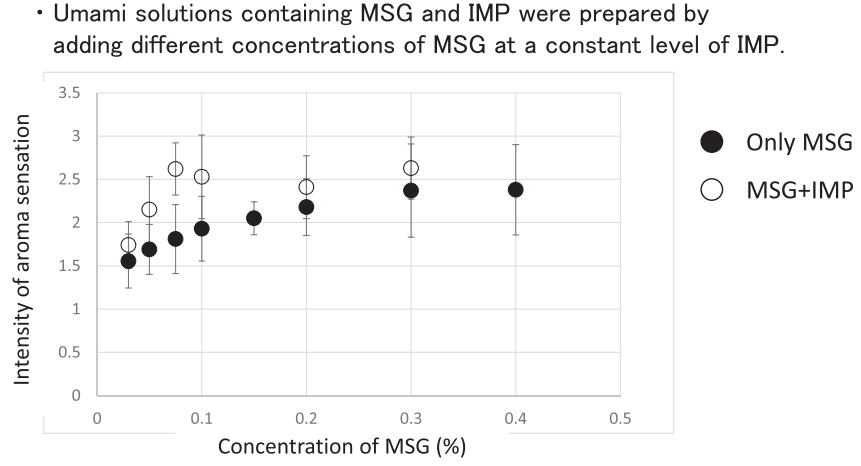


Fig. 1.8 Effects of umami compounds on the intensity of the aroma sensation, using a flavored solution

There have been some research studies on compounds involved in the mouthfulness *koku* attribute. Ueda et al. (1990) found that addition of an aqueous extract of garlic to soups enhanced the sensations of continuity, mouthfulness, and thickness in the soups. This extract was also found to have this enhancing effect when it was added to an umami solution composed of 0.05% MSG and disodium inosinate. The key compounds in the aqueous extract of garlic were clarified to be alliin, S-methyl-L-cysteine sulfoxide, and γ-L-glutamyl-S-allyl-L-cysteine. Furthermore, the addition of alliin at 0.05% (w/v), which had no aroma or taste, was reported to have this enhancing effect on the soups. Ueda et al. (1994) also clarified that trans-S-propenyl-L-cysteine sulfoxide (PeCSO) or its γ-glutamyl peptide (γ-Glu-PeCSO) enhanced the sensations of continuity, mouthfulness, and thickness in an umami solution when added in a concentration of 0.02% (w/v), although these compounds at this concentration did not add any aroma or taste to the umami solution.

Dunkel and Hofmann (2009) reported that addition of a nearly tasteless aqueous extract isolated from beans to a model chicken broth enhanced the sensations of mouthfulness and complexity, and successively induced a long-lasting savory taste on the tongue. They clarified that γ-L-glutamyl-L-leucine, γ-L-glutamyl-L-leucine, and γ-L-glutamyl-L-cysteinyl-β-alanine were the key molecules, and they called them *kokumi* peptides. Then, they clarified that γ-L-glutamyl peptides such as γ-glutamyl-Glu and γ-glutamyl-Gly, found in Gouda cheese, were key compounds that enhanced the mouthfulness and lingeringness of the matured cheese. Kuroda et al. (2013) discovered the *kokumi* peptide γ-L-glutamyl-L-valyl-glycine (γ-EVG), which is found in soy sauce, raw scallop, and processed scallop products. These *kokumi* peptides are perceived by calcium-sensing receptors (CaSRs) (Ohtsu et al. 2010). However, there has been no clarification of the mechanisms involved in the sensation of mouthfulness and lingeringness conveyed by the binding of *kokumi* peptides to CaSRs.

Ogasawara et al. (2006a, b) found that Maillard peptides with a molecular weight of 1000–5000 Da from soybean paste enhanced the sensations of lingeringness and mouthfulness in an umami solution and consommé soup when these peptides were added to these solutions. Maillard peptides, which are increased during fermentation in the production process of aged miso, were thought to be key substances in enhancing the characteristic flavor of miso.

Kurobayashi et al. (2008) reported that addition of three phthalides—sedanenolide, 3-n-butylphthalide, and sedanolide—to chicken broth enhanced the umami intensity, as well as the complexity, of the broth when these phthalides were added to the broth at the lower concentration than threshold level. Thus, although some substances have been discovered to possess the enhancing effect of mouthfulness, the enhancing mechanisms by these compounds were not clarified.

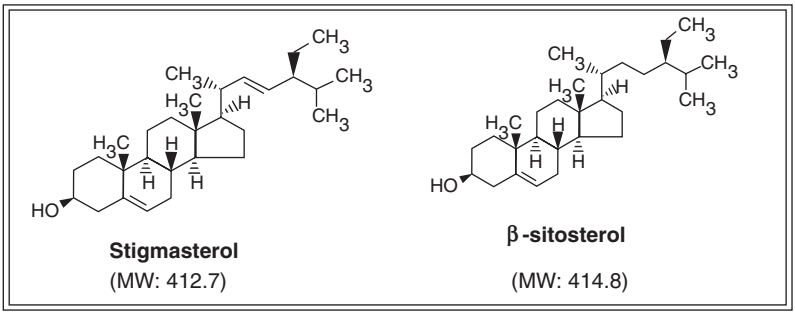
1.3.3 Lingeringness (Continuity)

The lingeringness in *koku* attribute of foods such as curry and stew is sensed in the whole oral cavity and in the nose as a retronasal aroma.

The mechanism accounting for the lingeringness of umami substances sensed in the oral cavity and the mixture of umami substances and *kokumi* peptides or Maillard peptides are still not clearly understood. When an umami compound is put into the mouth, the tactile stimulation of the tongue continues for a long time. Although sulfur-containing compounds or *kokumi* peptides also show the effect of lingeringness, there is little evidence that only these substances can show the same effect that umami compounds do.

Another example of the lingeringness *koku* attribute is caused by the binding activity of aroma compounds with lipids. Ramen noodles in pork bone soup and marbled beef from Japanese black cattle are rich dishes because of the lipid or fat content in these foods. Although pure lipids or fats have no taste and aroma in general, we can sense a complex taste and aroma when we put cooked lipids or fats into our mouth. Nishimura et al. (2016b) clarified that this phenomenon is brought about by taste and aroma compounds attached to the lipids and fats (Fig. 1.9). They found that a precipitate of heat-treated onion concentrate (HOC) interacted with aroma compounds and thus enhanced aroma persistence, including the lingeringness of the aroma, as a *koku* attribute of consommé. The key compounds in the precipitate were phytosterols—that is, β-sitosterol and stigmasterol. To my knowledge, this was the first study to show that phytosterols can interact with aroma compounds in foods. Lipids or fats in foods contribute to complexity and lingeringness as *koku* attributes

1. Stigmasterol and β–sitosterol in precipitate of onion juice concentrate were identified by pyrolysis GC/MS.
2. They can hold aroma compounds make us lingeringness of aroma sensation.



(Nishimura T. et al, "Phytosterols in onion contribute to a Sensation of lingering of aroma, a *koku* attribute", *Food chemistry*, **192**, 724 (2016))

Fig. 1.9 Compounds in precipitate of onion juice concentrate

of foods. It is well known that the presence of lipids influences the persistence of aroma compounds in foods. Aroma compounds in foods partially bind to lipids or fats by hydrophobic interaction.

1.4 Regulation of the Intensity of *Koku* Attributes in Food Development

To develop new food products possessing *koku* attributes, first an image of the sensations of foods should be created (Fig. 1.10). For example, we may want to produce foods with weak lingeringness and strong complexity and mouthfulness, or foods with strong complexity and lingeringness. To regulate the intensity of *koku* attributes, the methods described in Sects. 1.4.1, 1.4.2, and 1.4.3 seem to be effective.

1.4.1 Production of Foods Possessing Koku *Attributes*

As mentioned earlier, the complexity of *koku* attributes in foods is mainly produced by heating, fermentation, or conditioning. The intensity of the complexity in *Koku* attributes of these foods is regulated by the duration of these production processes.

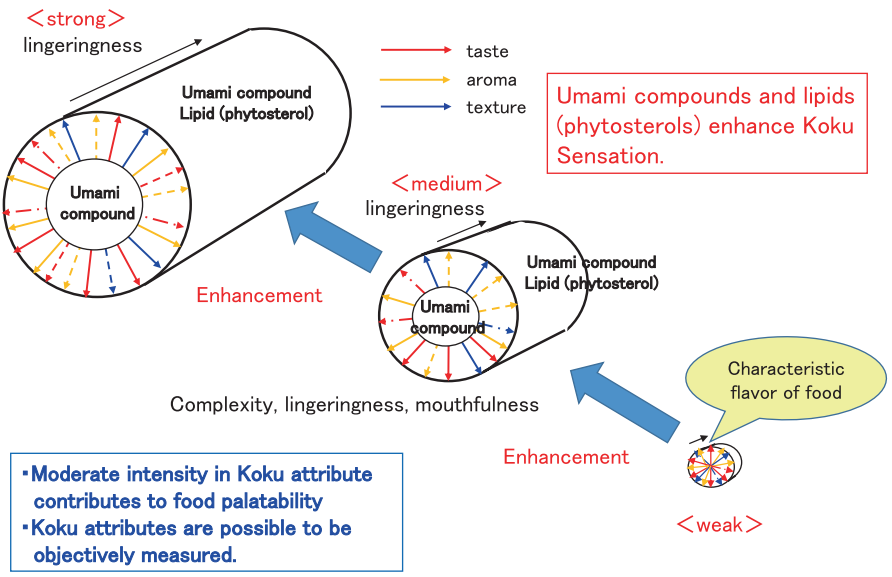


Fig. 1.10 Intensity of *koku* attributes

Bouillon is prepared by heating meats and various vegetables—such as carrot and onion—in soup for long time. During this heating process, free amino acids, sugars, and organic acids are extracted from the foodstuffs, and various favorable aroma compounds are also produced in the soup by the Maillard reaction between amino acids and sugars. The intensity of the complexity of the *koku* attributes of this bouillon is regulated by changing the duration of heating. In general, the complexity of these foods becomes stronger when they are heated for a long time rather than for a short time, because the extraction of taste compounds with heating increases with time.

Natural cheeses and some meats are conditioned for a long time at a certain temperature to produce favorable flavors in these foods. During conditioning of natural cheeses and meats, many kinds of taste compounds, such as free amino acids and peptides, are produced by endogenous or microorganism proteases, which enhance the complexity of the *koku* attributes of these foods. In the case of cheeses, specific aroma compounds are produced from fatty acids by the action of specific microorganisms. This complexity becomes stronger when they are conditioned for a longer time. In the case of meats, meaty and specific aromas of meat are produced by a Maillard reaction when the meats are heated. Umami compounds also increase during conditioning, leading to enhanced mouthfulness and lingeringness (Fig. 1.11).

Miso paste is produced by fermentation using soybeans. As the fermentation of the miso paste proceeds, the color of the paste changes from yellow to brown because of the Maillard reaction. During fermentation of miso paste, various kinds

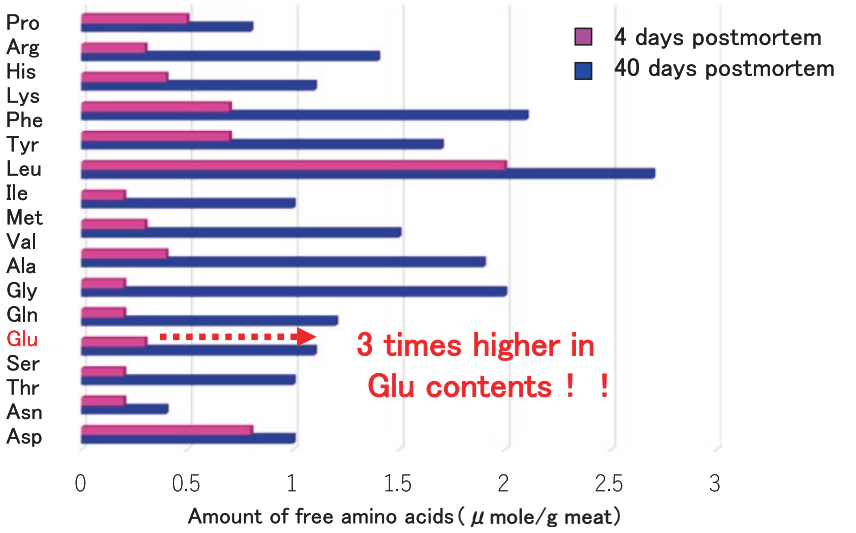


Fig. 1.11 Increase in free amino acids during postmortem conditioning of the loin of Japanese black cattle

of taste compounds—such as free amino acids, peptides, and aroma compounds—are produced and increased by the action of microorganisms. The incrementation of taste and aroma compounds causes complexity in the *koku* attributes of miso paste. Furthermore, incrementation of umami compounds and Maillard peptides during fermentation also occurs and enhances the mouthfulness and lingeringness of miso paste. Thus, lengthy fermentation of miso paste produces stronger *koku* attributes than brief fermentation.

1.4.2 Application of Substances with Enhancing Effects on Koku *Attributes*

When we increase the intensity of mouthfulness and lingeringness as *koku* attributes of foods, umami substances, both umami substances and *kokumi* substances, or both umami substances and Maillard peptides should be added to foods that have weak *koku* attributes. We examined the effect of the amount of umami compounds added to pork sausages on the *koku* attributes of this food. The addition of umami substances to sausages enhanced the intensity of the sensation of a retronasal aroma, the complexity of the sausage flavor, and the lingeringness of the taste.

We clarified that the addition of lipids to foods can enhance the intensity of the *koku* attributes of foods. First, β-sitosterol, which has the ability to bind to aroma compounds was added to Chinese soup, and the intensity of the soup's spicy aroma was enhanced (Figs. 1.12 and 1.13). The intensity of the lingeringness of the aroma and the complexity and mouthfulness of the soup were also enhanced. We also clarified that the addition of fat to pork sausages enhanced the intensity of the mouthfulness and complexity of the sausages.

1.4.3 Combinations with Foods Possessing Koku *Attributes*

In Japan there are delicious foods such as sashimi (sliced raw fish), *basashi* (raw horse meat), and *moro-kyu* (cucumber with *moromi* miso paste). Raw fish, raw horse meat, and cucumber are foods with no *koku* attributes. However, we eat raw fish dipped in soy sauce, raw horse meat dipped in *tare* sauce (a special sauce), and raw cucumber with miso paste put on its surface. Soy sauce, *tare* sauce, and miso paste are foods with *koku* attributes. Even if there are no *koku* attribute in foods, we can change these foods into foods that do possess *koku* attributes by combining them with other food materials such as soy sauce and miso paste.

Japanese soup stock (dashi) is made with dried seaweed (kombu) and bonito shavings, and it has weak *koku* attributes because it contains umami substances. Some Japanese chefs say that the addition of small dried sardines and mackerel shavings to dashi can make its *koku* attributes much stronger.

Thus, we can regulate the intensity of the *koku* attributes of foods by combination with food materials that have stronger *koku* attributes.