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Challenges in the use of natural flavorings and labeling compliance in meat preparations in the Umbria region (central Italy)

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Abstract

There is an increasing trend among food business operators to use natural flavorings in meat preparation to reduce microbial spoilage and extend the shelf life. The objective of this study was to evaluate the prevalence of the use of natural flavoring mixtures in meat preparation, the possible presence of additives in natural flavorings, and the assessment of any transfer of additives contained in natural flavorings into experimentally prepared beef burgers.

Based on a preliminary survey conducted as part of the study, approximately 87% of establishments used natural flavoring mixtures in meat preparation. The same natural mixtures used by local industries were added to minced meat in order to produce experimental hamburgers.

Citric acid was found in both natural flavoring mixtures ranging from 1760 mg/kg to 92,731 mg/kg and experimental burgers ranging from 57 mg/kg to 2248 mg/kg. Ascorbic acid was present in all natural flavoring mixtures from 260 mg/kg to 98,858 mg/kg but was recovered in three burgers up to 1075 mg/kg. Acetic acid was found in three flavoring mixtures (range 23,539-77,421 mg/kg) and transferred to three treated burgers (range 3063-3202 mg/kg). Nitrite was never found in hamburgers and only once in a natural flavoring mixture at a dose of 26 mg/kg, whereas nitrate was found in some natural flavoring mixtures up to 788 mg/kg but never in hamburgers.

The study has raised some critical issues about the appropriate and conscious use of natural flavorings and the possible inadequate meat preparation labeling in the Umbria region.

Introduction

Meat preparations such as hamburgers are highly appreciated by consumers for their easy preparation and versatility in consumption, considered time-saving strategies in modern lifestyles (Branciari *et al*., 2017). However, the meat grinding and manipulation process leads to a more perishable product (Roila *et al*., 2022) prone to oxidative damage due to the release of oxidation-sensitive lipids together with pro-oxidative factors (*e.g*., Fe-, Cu-ions). Oxidation of meat can cause discoloration, off-flavors, nutrient depletion, drip loss, and the formation of toxic compounds (Falowo *et al.*, 2014).

The use of additives in meat preparations is currently regulated by EC Regulation 1333/2008 and following amendments (European Parliament and Council of the European Union, 2008). Meat preparation as defined by EC Regulation 853/2004 is considered as unprocessed meat in which only a restricted number of additives are authorized (European Parliament and Council of the European Union, 2004a). Accordingly, there is an increasing trend among food business operators (FBO) to use natural substances in meat preparation to improve oxidative stability, reduce microbial spoilage, extend the shelf-life, prevent nutritional losses, and improve health outcomes such as aging, cancer, and cardiovascular diseases (Jiménez-Colmenero *et al*., 2001; Carocho *et al.,* 2018). Studies demonstrate that the use of natural substances can help prevent or minimize oxidation in muscle foods (Rodríguez-Carpena *et al*., 2011; Kim *et al*., 2013). Indeed, natural substances have been studied for their ability to prevent oxidation and negative quality changes in raw meat, freshly prepared meat, and meat preparations (sliced or comminuted meat) during processing and storage (Ahmad *et al.*, 2015; Roila *et al.*, 2016; Roila *et al.*, 2022). The plant source, whether a spice, herb, essential oil, crude extract, or phenolic-rich concentrated extract, can provide numerous bioactive compounds albeit they must be compatible with the meat system in terms of sensory coherence and effectiveness under specific processing conditions (Carocho *et al.*, 2018).

Several studies have explored the potential use of natural flavorings in the meat industry, assuming they are effective alternatives to conventional additives.

Nevertheless, there is a debated issue concerning the possibility of adding these natural compounds to ground meat preparations produced in cutting plants connected to a local retail establishment (Varvara *et al*., 2016). The most debated question regards the possibility of admixing natural flavoring mix to minced meat preparations. In particular, it is crucial to define whether the presence of additives in an intermediate food ingredient is permissible or not and if its presence needs to be included in the ingredients list.

The objective of this study was to assess, in facilities registered under EC Regulation 852/2004 and recognized under EC Regulation 853/2004 in the Umbria region, the prevalence of the use of natural flavoring mixtures in meat preparation; the possible presence of substances identifiable as additives in natural flavoring mixtures; the verification of the correct declaration of the presence of additives in labeling and the assessment of any carry-over of additives contained in the natural flavoring mixture to experimentally prepared meat preparations.

Materials and Methods

In the initial phase of the project a recognition of the meat handling facilities was conducted through the Veterinary and Food Information System. In particular, the census covered the minced meat and meat preparations establishments approved in accordance with Regulation (EC) 853/2004, as well as meat processing and handling establishments that were registered in compliance with Regulation (EC) 852/2004 from a specific area of the Umbria region (European Parliament and Council of the European Union 2004a, 2004b).
Subsequently, a survey was developed with the aim of highlighting the prevalence of using natural flavoring substances in meat preparations and meat products by local facilities and examining the variety of extracts used in both products.
A questionnaire was administered to 15 establishments that were deemed to be representative of the 99 meat processing establishments that were registered and recognized in the Umbria region. The participants were provided with written consent prior to their participation in the study. The sample size was determined using the formula for estimating prevalence, assuming an expected prevalence of 50% with 95% confidence level and a precision of 24% corresponding to effect size. The formula used for calculating the adequate sample size in a prevalence study is $n=Z^2P(1-P)d^2$ where n is the sample size, Z is the statistic corresponding to the level of confidence, P is the expected prevalence and d is precision (Daniel, 1999).
The questionnaire included the following information regarding meat preparation: type of products (prepackaged or unpackaged meat preparation); *yes/no* use of a natural flavoring substance in meat preparation; the FBO reason for using flavorings in meat preparation: a) to extend the shelf life of the product, b) to improve meat preparation appearance, c) to improve meat preparation flavor, d) without any particular reason; yes/no the producer's awareness of natural flavoring mixture composition. In *Supplementary Table 1*, we provide an overview of all information reported in the questionnaire.

Chemical analysis of additives in flavoring substances and meat preparation

The second phase of the research involved the evaluation of the presence of additives in 6 natural flavoring mixtures (NFMs) (Figure 1) used in meat preparation by targeted manufacturers and the carry-over of additives possibly contained in NFMs to experimentally formulated meat preparations.
Each NFM and the experimental burger has been tested for the presence of the following additives: nitrate, nitrite, sulfites, ascorbic acid, citric acid, acetic acid, carminic acid (E 120) and nine synthetic food dyes (Tartrazine - E 102; Quinoline yellow - E 104; Sunset Yellow - E 110; Azorubine - E 122; Amaranth - E 123; Ponceau 4R - E 124; Erythrozine - E 127; Red 2G - E 128; Allura Red - E 129).
For the analysis of additives in the two matrices, NFMs and meat preparations, the same sample treatments were performed and the determinations were carried out in triplicate.
Nitrate and nitrite extraction and quantification were carried out via high-performance ion exchange chromatography using a chromatography system (Thermo scientific Dionex ICS 5000^+ EG, Sunnyvale, CA, USA) following the procedure of Haouet *et al.* (2017), Roila *et al.* (2018) and Iammarino *et al.* (2017).
The determination of sulfites involved an initial extraction phase: 10 grams of the homogenized sample were mixed with 50 ml of ultrapure water and 30 mL of 3% H_2O_2 and then a subsequent

distillation with 30 mL of 3.7% hydrochloric acid. Finally, the extract was filtered and analyzed using ion exchange chromatography with conductivity detection. The chromatographic separation of sulfite was obtained using a IonPac AS11-HC (250×4 mm) column, a flow rate of 0.9 mL min^{-1} and a run time of 15 minutes.

For acetic acid, the extraction phase was conducted as follows: 20 grams of homogenized sample were added to 100 mL of 0.1N H_2SO_4, agitated on a mechanical shaker for 1 h, and centrifuged at 4000 rpm at 4°C for 15 minutes. The supernatant was then filtered through a Whatman 41 paper filter, through a 0.45 µm nylon filter later, and injected into high-performance liquid chromatography (HPLC) for analysis. HPLC analysis was carried out isocratically with a 300×7.8 mm i.d. cation exchange column held at 50°C, at a flow rate of 0.7 mL min^{-1} and using 8 mM sulfuric acid as mobile phase.

For citric acid, a 2-g portion of the homogenized sample was mixed with 20 ml of ultrapure water. After centrifugation for 10 minutes at 4500 rpm at 4°C temperature, the extract was first filtered through paper (Whatman 41) and through a 0.45 µm syringe nylon filter later, prior to ion-exchange chromatographic analysis. The conditions of the chromatographic method applied were a IonPac AS11-HC (250×4 mm) column, a flow rate of 1 $mLmin^{-1}$ and a run time of 15 minutes.

Ascorbic acid was detected following the procedure of Maoloni *et al.* (2021). Briefly, the extract was filtered through a 0.45 m syringe nylon filter and analyzed by HPLC with a UV-diode array detector (UV-DAD) detector (SHIMADZU Nexera X2, Milano, Italy) using an RP-C18 column (250×3.0 mm i.d., 5 m), a run time of 15 minutes and 245 nm absorbance. Chromatographic separation was performed at an isocratic mobile phase of 0.04 mol L−1 phosphate buffer, pH 2.8, with a 0.34 mL min^{-1} flow rate and an injection volume of 20 µL.

Carminic acid was determined following the procedure of Scotter (2011). Five sample grams were mixed with 15 mL of 2N HCl and sonicated in an ultrasonic bath for 10 minutes at 60°C temperature; each sample was centrifuged, and the supernatant was collected in a 50 mL flask. Following a two times repetition, the supernatants were collected in the same 50 ml flask and the extract was analyzed by HPLC with a UV-DAD detector (SHIMADZU Nexera X2, Milano, Italy) using a Gemini-C18 column (150×4.6 mm), a run time of 20 minutes, a flow rate of 0.4 mL min^{-1} and 495 nm absorbance.

The other nine synthetic food dyes were analyzed following the method of Bonan *et al*. (2013). One g of sample was extracted with 75% ethyl alcohol and 1% NH_3, heated in an 80°C water bath, sonicated and mixed; the extraction was repeated two times, collecting the supernatant, adjusting pH at 2 and filling to 50 mL; after solid phase extraction, the purification was performed using a Discovery DPA 500 mg/6 mL tube, and detection was carried out by HPLC with a UV-DAD detector (SHIMADZU, Nexera X2, Milano, Italy), using a Luna-C18 column (150×3 mm), 20 µL loop injector, 0.65 $mLmin^{-1}$ flow rate and a 30 minutes elution gradient.

Experimental burger production

Six experimental burgers were produced in a pilot plant of *the Istituto Zooprofilattico Sperimentale* of Umbria and Marche Regions, with the six different NFMs used in the Umbria region. The burgers were produced according to the recipes (burger recipes are reported in *Supplementary Table 2*) proposed by the 15 establishments under investigation. Each establishment provided the meat used for the burgers. The meat was ground in a professional meat grinder equipped with a 4 mm-hole plate and the ground meat was divided into six different formulations, each 5 kg weight, and aseptically hand-mixed with the ingredients and flavoring mix of each recipe, a control batch was also produced without the mix addition. Finally, the minced meat added with specific NFM was shaped in burgers (about 120 g each) and subsequently placed in a display refrigerator at 4±2°C for 7 days, under alternating exposure to fluorescent light (12 h light/12 h darkness) to simulate retail storage conditions.

The composition of the NFMs of the 6 recipes are reported in *Supplementary Table 3*. Each of the six experimental hamburger formulations, as well as the control group, underwent chemical analysis for additives that may be present in the natural flavoring.

Results and Discussion

The results of the survey reported in Figure 2 show that 13/15 (87%; 95% confidence interval: 62-96%) of the interviewed establishments use NFMs in meat preparation and meat products (Figure 2). The survey highlights that in the majority of the establishments (8/15-53%) meat preparations are sold unpackaged.

According to the survey and considering the possibility of giving more than one answer, 54% (7/13) of the FBO uses NFMs in meat preparation both *"*to extend the shelf life of the product and "to improve its flavor"; 38% (5/13) "to improve its appearance*"*, and 23% (3/13) *"*without any particular reason*"* (Figure 2). Based on the responses, it appears that such products are used for technological purposes.

The composition of the NFMs used is known by 54% (7/13) of the establishments surveyed but 46% (6/13) of FBOs uses these substances in their preparations without knowing their composition. As reported in the literature (Goodman, 2017), the first issue for the natural flavouing mix use is if the natural mix flavoring's composition complies with Regulation 1334/2008. Furthermore, it is possible for natural flavoring to contain artificial or natural preservatives. To date, it is unclear if all incidental additives are exempt from labeling requirements or may fall outside the bounds of circumscribed natural regulations (Godmann, 2017). This is of crucial significance as the term natural on food labels could be an important attribute that influences consumers' purchase decisions (Hung *et al.,* 2016).

The results of the additives' presence in NFMs (mg/kg) are reported in Table 1.

Table 1 shows that all the tested NFMs contain both citric acid and ascorbic acid. Acetic acid was present in three NFMs. Traces of nitrite were detected in one NFM and nitrates were found in four of the six NFMs tested. Sulfites, carminic acid, and coloring agents were not detected in any of the NFMs.

Numerous natural extracts have been applied to meat and meat products, with herbs and spices being the most used as clean-label alternatives to nitrites and sulfites (Oswell *et al.*, 2018). However, there are several plants, such as spinach, radish and celery, that contain more than 2500 mg nitrate/kg (Roila *et al.*, 2018), and their extracts can be used as natural sources of nitrate in meat products. However, nitrates of natural origin have no health-related advantage compared to synthetic ones, and they only provide a clean label option for the consumer. On the other hand, plant and fruit extracts such as citrus, grapes, pomegranates and apples are currently receiving attention as sources of natural antioxidants and antimicrobials with potential value for extending the shelf-life of meat without health risks (Simitzis and Deligeorgis, 2018). As noted by Godmann (2017), the flavor industry comprises a small elite group of scientists who specialize in developing flavors. This industry is remarkably secretive, and as previously mentioned and illustrated, natural flavoring mixes may contain synthetic preservatives that are not always disclosed in the technical sheets or that are disclosed in the technical sheet but are not provided to the FBO. In this scenario, the potential presence of additives in the finished food, through the carry-over principle, may not be allowed or may be omitted. Therefore, relying solely on natural flavoring mixes may be misguided because the term is not strictly regulated.

Citric acid and ascorbic acid detected in three of the NFMs were not indicated on the label and in the technical data sheet. Three products contained citric acid, acetic acid, and ascorbic acid additives listed in the technical data sheet and in labeling. Citric acid was found in natural NFMs ranging from 1760 mg/kg to 92,731 mg/kg. Ascorbic acid was present in all NFMs from 260 mg/kg to 98,858 mg/kg, Acetic acid was found in three NFMs ranging from 23,539 to 77,421 mg/kg. Nitrite was in only one NFM at a dose of 26 mg/kg, whereas nitrate was found in four NFMs up to 788 mg/kg.

EC Regulation 1333/2008 and EC Regulation 601/2014 allows the use of ascorbic acid, citric acid and acetic acid as *quantum satis* in meat preparation, but EU Regulation 1169/2011 requires a mandatory declaration to ensure the appropriate level of consumer information (European Parliament and Council of the European Union, 2011). The carry-over principle is a concept used in food regulation (European Parliament and Council of the European Union, 2008, 2011) to determine whether certain ingredients or additives must be declared on the label. In this context, food additives and food enzymes, whose presence in a given food is solely due to the fact that they were contained in one or more ingredients of that food, in accordance with the carry-over principle referred to in points (a) and (b) of Article 18 (1) of EC Regulation 1333/2008, provided that they serve no technological function in the finished product. This principle is based on the understanding that the additive is present in trace amounts and does not have a significant impact on the final product. In the present study, the level of the additives has a controversial aspect since the high concentration in meat preparation can indicate a technological purpose, such as preservation or coloring, and must then be declared in labeling, conversely not.

Table 2 shows the results of the amount, expressed in mg/kg, of additives in burgers formulated with six different recipes with the addition of six NFMs.

According to Table 2, in some cases, experimental burgers contained additives that were also present in the NFMs used to prepare them, proving that the carry-over occurred. No additives were detected in the control batch formulated without NFM. Citric acid, detected in all NFMs, was recovered in all burgers, whereas ascorbic acid, which was present in all NFMs, was transferred only to three out of six burgers. Acetic acid present in three NFMs was also recovered in the three burgers ranging from 3063 to 3202 mg/kg. Citric acid was found in experimental burgers ranging from 57 mg/kg to 2248 mg/kg. Ascorbic acid was transferred to three burgers ranging from 739 to 1075 mg/kg. Nitrite and nitrate were never found in burgers.

The initial draft of EC Regulation 1333/2008 envisaged the possible addition of certain additives only for "prepacked preparations of freshly minced meat" (European Parliament and Council of the European Union, 2008). Subsequent amendments were made by rewording Annex II of EC Regulation (EC) 1333/2008, allowing the use of additives for "only gehakt, prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added" (European Parliament and Council of the European Union, 2008). Furthermore, it corrected Article 18 and established that the carry-over principle could be applied to meat preparation. As a result, the presence of an unauthorized food additive shall be permitted when food flavoring has been added, where the food additive has no technological function in the final food as previously stated.

The results of these experimental tests demonstrated that the natural flavoring in meat preparation was rather used for technical purposes, as stated in the survey.

Any failure to declare additives in the labeling of NFM results in an unconscious carry-over of the same into meat preparations, resulting in labeling lacking such information for customers.

Conclusions

The current study, conducted in the Umbria region about the use of NFMs in meat preparations, raised some critical issues. The use of natural flavorings in meat preparations is a widespread practice in the region, as evidenced by the survey and FBOs who use NFMs are not fully aware of their composition. Additives are present in the natural flavoring mixes analyzed and, in some cases, are transferred to the final product. Furthermore, the survey highlights that the purpose for the addition of the natural flavoring mix in meat preparations is frequently technological. In this case, however, the use of each additive present in the natural mix must be authorized and, to properly inform consumers, indicated in the label. On the contrary, food additives whose presence in a given food is solely due to the fact that they were contained in one or more ingredients, in accordance with the carry-over principle, and serve no technological function in the finished product can be omitted in the label.

Then an intentional addition of mixtures of natural flavoring containing additives for technological purposes requires mandatory declaration in labeling by current legislation.
These critical issues highlight the need for increased awareness among FBOs about the appropriate and conscious use of NFMs, with a focus on consumer safety.

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Online supplementary material

Supplementary Table 1. Questionnaire administered to establishments.
Supplementary Table 2. Experimental burger recipes.
Supplementary Table 3. Natural flavoring composition declared in THE technical sheet.

Table 1. The concentration of additives in the 6 natural flavoring mixes examined. Results are expressed as mg/kg of mixture (mean ± standard error).

Natural flavoring	Ascorbic acid	Citric acid	Acetic acid	Sulfite	Nitrite	Nitrate	Carminic acid	9 synthetic food dyes
Mix 1	39,445±0.6	92,731±35	77421±20	<10	<10	<30	<10	<10
Mix 2	260±0.0	1760±4.3	<10	<10	26±0.0	114±0.0	<10	<10
Mix 3	1653±0.3	4039±9.2	<10	<10	<10	156±7.8	<10	<10
Mix 4	54,868±0.3	53,190±4.3	23640±3.5	<10	<10	771±3.8	<10	<10
Mix 5	4609±0.6	2612±1.5	<10	<10	<10	788±8.9	<10	<10
Mix 6	98,858±0.7	41,352±35	23539±4.4	<10	<10	<30	<10	<10

MIX, commercial natural flavoring mixture.

Table 2. The concentration of additives in the six experimental burgers. Results are expressed as mg/kg of meat (mean ± standard error)

Burger formulation	Ascorbic acid	Citric acid	Acetic acid	Sulfite	Nitrite	Nitrate	Carminic acid	9 synthetic food dyes
Burger 1	1075±1.5	2248±0.3	3202±0.4	<10	<10	<30	<10	<10
Burger 2	<10	235±1.2	<10	<10	<10	<30	<10	<10
Burger 3	<10	268±0.7	<10	<10	<10	<30	<10	<10
Burger 4	739±1.7	790±0.9	3063±0.8	<10	<10	<30	<10	<10
Burger 5	<10	57±0.3	<10	<10	<10	<30	<10	<10
Burger 6	873±0.9	474±0.9	3199±1.1	<10	<10	<30	<10	<10

Burger formulations: the number after each burger indicated the different recipe [*e.g.*, Burger 1 obtained with minced beef meat and mix 1 (commercial natural flavoring mixture 1), Burger 2 obtained with minced beef meat and mix 2 (commercial natural flavoring mixture 2), *etc.*]

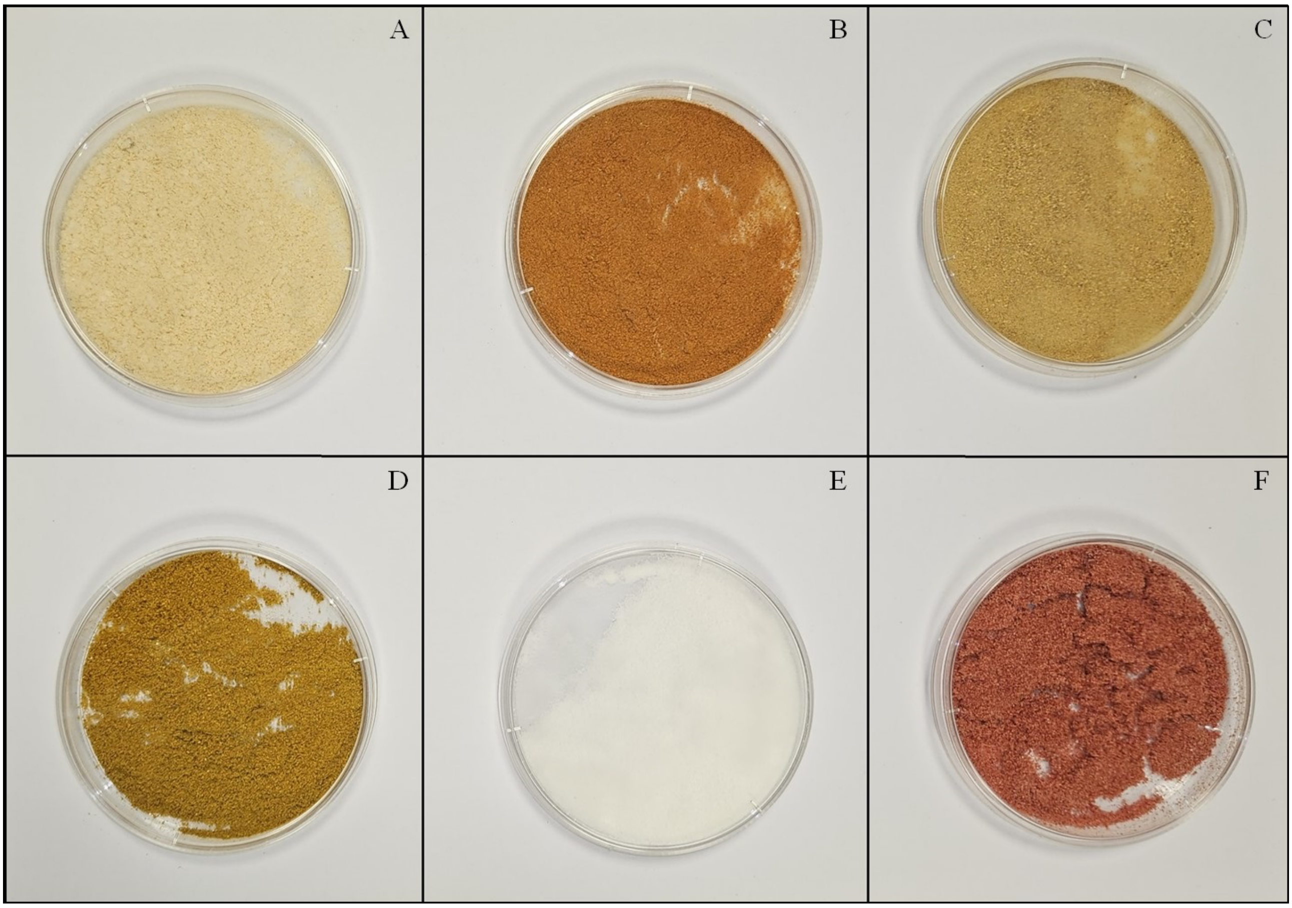

Figure 1. **Natural flavoring mixtures. A) Mix 1; B) mix 2; C) mix 3; D) mix 4; E) mix 5; F) mix 6.**

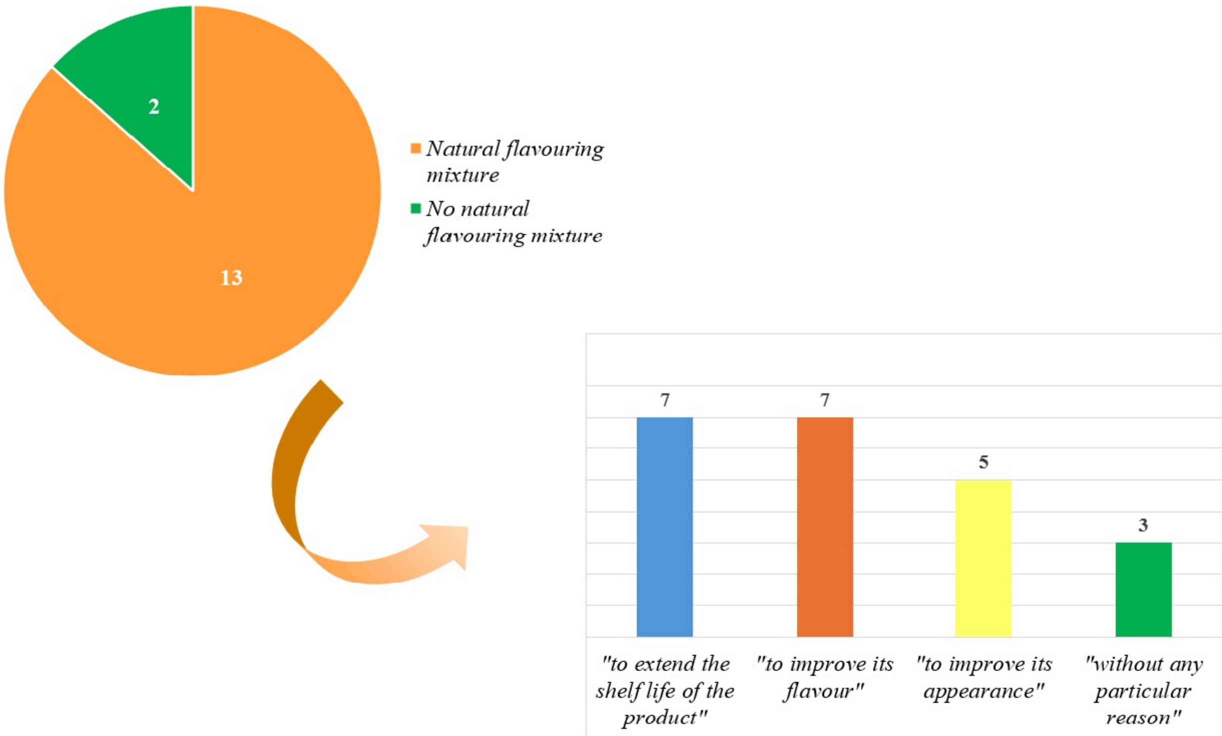

Figure 2. Prevalence of establishments using natural flavoring mixtures and their purpose.