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THESIS BOOK

THE ROLE OF ACTIVE MATERIALS OF ROSEMARY IN SUNFLOWER OIL

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1. BACKGROUND

The application of seasonings and herbs is one of the most obvious possibilities of product-development in the food industry. Spices and herbs are those plant materials which are minimally processed (mainly dried and cut) and applied in foods for improving their organoleptic values. In European cuisine the green herbs are typical for this purpose even if the spices from Far-East are well known since ancient times. There are textual documents from these ages e.g. : Apicis: De re coquinaria (cited by ROMVÁRY, 1974). Mediterranean climate especially advantageous for growing fragrant green herbs and this is utilized by the people of the region. These herbs- e.g. rosemary, basil, lavender etc.- are mainly members of the Labiatae plant family. From the Mediterranum they spread quickly around Europe, or at least applied in the gastronomy. The herbs are used not only in dishes but for pourable sauces too. Special field of application is the flavouring the edible oils. However different types of olive oils (extra virgin, sansa etc) are typically used for this food items, but nowadays some other flavoured edible oils such as corn oil, grape seed oil and sunflower oils are available in food market. The oils are particularly salad oils, but there are examples for the application in sauces and spreads. Of the home producers the products of Bunge Co. and Rebiol Ltd. are known. These flavoured oils contain sunflower oil and spice extracts.

Flavouring of oils basically follows two ways: applying oleoresins and applying immersion technology.

Oleoresins are mixture of volatile and non-volatile extract of a spice. Volatiles are gathered by steam distillation, non –volatiles by solvent extraction. The solvent must be removed from the extract. However the oleoresins are standard products the problem of the solvent residues means some difficulties in application. Additionally oleoresins are still expensive even for big companies.

Immersion means the dipping of the herb in the oils for some period of time during that the volatile and non-volatile components should diffuse into the oil. The basic idea of this method is rather simple and it should consider as a traditional way of flavouring. There are only few scientific efforts to study the characteristics of the immersed oils (e.g.: DeFelice et al. 1993). The main objective of the present work is to analyze this lesser known product.

Far from the scientific point of view there is some practical interest in application the immersed oil in food production.

In the experimental design my aim was to apply well known materials: sunflower oil and dried rosemary leaves. Both are easily available in the Hungarian food market and consumed frequently. Based on my previous research I aimed to apply objective methods in describing and characterizing the development of the aroma in the oil. For these purpose not only human sensory investigations but instrumentals measurements were planned partly by SPME GC MS technique, partly by electronic nose chemosensor array. This letter sufficiently mimics the human sensation.

Beside the first aim it was purposed to establish the oxidative stability of the flavoured oil.

Thirdly the aromatizing effect was aimed to study by frying tests.

The fourth purpose was to establish the heat-stability of the aromatized sunflower oil.

The whole work, including measurements, and discussion followed this four general aims.

As an important point of view the work tried to help the similar purpose investigations.

2. OBJECTIVES

Based on the available previous scientific results the general aims were detailed by the followings:
1: Describing the aroma development of rosemary immersed sunflower oil. Establish the correlation between the aroma and the amount of immersed rosemary and the length of time of immersion. For this purpose application of electronic nose chemical sensor array as well as human sensory evaluation and GC MS technique. Suggestions for the appropriate parameters of dipping as a result of the measurements .

2: Establish the oxidative stability of the rosemary flavoured sunflower oil. Application for this purpose the widely used Rancimat-test and measure the amount of the cinnamic acid derivatives in the oil which are considered as the main group of the antioxidative compounds of the rosemary.

3: Establish the aromatization effect of the flavoured sunflower oil. For this purpose organizing frying experiments with potato chips and pork loin as test materials. Sensory investigations in order to establish the most effective parameters of immersion in the point of view of aroma transfer.

4: Analyzing the heat stability of the flavoured sunflower oil. In this experiment those samples are involved that have effective aromatizing character. Heating the samples up to 180 °C for different period of time. Measurement of the primary and secondary oxidation compounds by peroxide number, acidity and anisidine number. Establish the amount of total polar compounds as a function of heating time. Analyzing the derivatives of the phytosterols of the flavoured and the control samples in order to establish the significance of the antioxidative compounds of rosemary in the sunflower oil.

Based on the above detailed four-part experiment establish the possible manufacturing criteria and the application in the food production.

3.MATERIALS AND METHODS

3.1 Materials

For the experiments commercially available sunflower oil (type Vénusz) was used . The oil was freshly produced and originated directly from the producer: Bunge Co. Hungary. As the herb, dried, cut rosemary leaves were collected from the retailer : Kotányi Hungária Ltd. The rosemary was Tunisian origin and the main characteristics were : water content :10 %, foreign materials : 0,1%, essential oils : 2,4 %.

3.2 Experimental design

For the above detailed four purposes the experiments were designed as follows .
For studying the aroma development rosemary leaves were immersed in 500-500 g sunflower oil by 0.5 %, 1.0%, 1.5% and 2.0% respectively. The immersion period of time was 28 days. Samples were packaged in PET bottles and stored at ambient temperature in light. The bottles were kept horizontally in order to avoid the separation of the leaves from the oil. Measurements were performed at the 1^{st}, 4^{th}, 7^{th}, 14^{th}, 21^{st} and at the 28^{th} day of immersion. There were individual samples prepared for these altogether 24 set points. Unflavoured sunflower oil was used as control and held under same conditions.
Samples were analyzed by human sensory panel as well as by means of electronic nose chemosensory array and by SPME GC/MS equipment.
Following the first experiment the same samples were analyzed for study the oxidative stability. For this purpose the widely used Rancimat test was applied. To complete the stability investigations cinnamic acid derivatives were measured to, since these compounds are considered as antioxidative materials of rosemary.
Based on the results of the first trial samples which have characteristic aroma were involved into the third experiment i.e.: studying the aromatization effect. From the selected samples 5-5 l new samples were prepared and used in frying tests. Potato chips and pork chop were fried in flavoured and in control samples at 180 °C and judged by sensory panel immediately after the treatment.
The heat stability of the flavoured samples was studied as follows. Samples which proved to have good aromatizing effect were selected and heated up to 180°C and kept at this temperature for 2 hours and 6 hours. Sensory properties of the heated samples were analyzed by electronic nose system. Further analytical measurements were performed to measure the primary and secondary oxidation processes : acidity, peroxide number and anisidine number. Total polar compounds were measured too. Of the valuable minor components of the oil, phytosterol derivatives were measured both in control and in flavoured samples.

3.3 Methods

In Table 1. are summarized the applied methods belonging to the research objectives:

Table 1: Summary of the applied methods

<table>
<tr><td rowspan="9">Methods</td><td colspan="4">Research objective</td></tr>
<tr><td>Aroma development</td><td>Oxidative stability</td><td>Aromatizing effect</td><td>Heat stability</td></tr>
<tr><td>Electronic nose system</td><td>Rancimat test</td><td rowspan="8">Human sensory judgments</td><td>Electronic nose system</td></tr>
<tr><td>Human sensory judgments</td><td rowspan="7">Cinnamic acid derivatives</td><td>Acidity</td></tr>
<tr><td rowspan="6">SPME GC/MS</td><td>Peroxide number</td></tr>
<tr><td>Anisidine number</td></tr>
<tr><td>Total polar compounds</td></tr>
<tr><td>Phytosterol derivatives</td></tr>
</table>

The four research objectives make necessary to organize partly correlated sequence of measurements. Firstly the investigations of aroma development were planned. This involved three different measurements. The objective sensory characteristic was analyzed by the electronic nose system. The results of the chemosensor array were completed by the human sensory investigations and finally the chromatography served to detect the presence of the main aromatic compounds in the oil.

The second objective of the work was to establish the oxidative stability of the flavoured sunflower oil. All the immersed samples as well as the control were involved to these investigations. Since Rancimat test is a widely used method in lipid science it was hoped to make possible comparison of the results with other similar investigations. The application of the measurement of the cinnamic acid derivatives served to investigate the significance of the antioxidative components of the rosemary in the sunflower oil.

Based on the results of the aroma development analysis was planned to determine the appropriate parameters of the immersion which have possible effect on the appearance of the aroma in the foods which are fried in the flavoured oil. The experiment tried to simulate the conditions of household frying. The fried foods were analyzed by human sensory tests.

Based on the results of the former experiment samples were chosen to the fourth trial. The aim was to analyze the heat stability of the samples which have good aromatizing effect. Two liters of these samples and the control was heated in household friteuse equipment (Tefal Versalio). The temperature was 180°C, the heating kept for 2 hours and 6 hours. Temperature and the time were controlled. The surface of the oil was 6.25 dm^2 , the volume 2 dm^3 . Consequently the surface: volume ratio is

3.125 dm^{-1}. Electronic nose system was applied to investigate the aroma modifications during heating since the possible undesirable volatiles should have make difficulties in objective human sensory judgment. Analytical measurements were applied additionally.

These were: acidity, peroxide number, anisidine number in order to follow the primary and secondary oxidation processes . Amount of total polar compounds were measured too. Of the valuable minor compounds of the sunflower oil phytesterols were measured. Beside the total amount of phytosterols, phytesterol derivatives were analyzed to establish whether the antioxidative components of the rosemary have protective effect on the sterols. The measurements were performed at the control samples too.

4. RESULTS AND DISCUSSION

4.1. Aroma development

The first objective of the present work was to describe the development and modification of the rosemary aroma in the sunflower oil. The results of the applied electronic nose system, human sensory evaluation and SPME GC/MS measurements concordantly proved that the aroma development during immersion can divide into two periods. The first period covers approximately the first two weeks of immersion and the second covers the remaining two weeks.

The appearance of the aroma was detectable at the fist day of immersion by means of the electronic nose system. The differences from the control were in correlation by the rosemary concentration, except the 0.5% samples. The human sensory panel detected the differences but only in case of smell. The judgment of the taste did not differ significantly from the control. These phenomena indicated that during the first period of the immersion the volatile components should have diffused into the sunflower oil. By the textual commentaries of the panel the aroma was characterized as “fresh”. The frequency of the freshness followed approximately the concentration of the rosemary. The 0.5 % samples were characterized mostly “neutral” and rarely “fresh”. Consequently this small amount of rosemary can cause special modification which is different from the oil but not characteristic to the herb.

This was in accordance with the SPME GC/MS measurements. The results of this showed that the dominant volatile compounds appear in the oil at the very beginning of the immersion but the amount of them are strongly correlated with the amount of the immersed rosemary. Consequently the different aroma of the 0.5 % samples originate from the small amount of the rosemary and not from other compound.

The electronic nose system detected a special modification in the aroma during the middle period of immersion. The tendency of the aroma development disappeared however the differences remained rather obvious. The human sensory panel described the samples ambivalently as “neutral”, “fresh” and “rosemary-like”. It was observable at least that the higher concentration samples showed stronger the smaller concentration mild aromas.

For the third and fourth week of the immersion the aroma showed new tendency. The 0.5% and the 1.0% samples formed a separate cluster and the 1.5% and 2.0% samples an other. The small concentration samples were described as “fresh” and “rosemary-like”, the higher concentration samples “rosemary-like” and “bitter” and fairly frequently “undesirable”. Regarding this period of immersion there was no new compound detectable by SPME GC/MS consequently new components did not diffuse into the oil from the rosemary. The undesirable flavour possible originated from the non volatile compounds which are located in the deeper tissues of the rosemary leaves.As a summary I could draw the conclusion that longer period of immersion did not cause stronger aroma in the oil. The intensity of the aroma can increase by the amount of immersed herb.

4.2 Oxidative stability

Oxidative stability was measured by means of Rancimat 743 apparatus. Results showed more-less increase of the oxidative stability comparing to the control. The induction time was obviously higher only in the case of the 1.5% and 2.0% samples for the last week of the immersion.
Based on the results I could calculate a two variable response surface. The exponential regression equation had close correlation between the induction time as dependent variable and the concentration and the length of time of immersion as independent variables. The values of the partial correlation coefficients proved that the concentration has stronger effect on the oxidative stability than the length of time of immersion.

The phenomena were confirmed by the results of the cinnamic acid derivatives measurements. These compounds were detectable in the oil only from the third week of the immersion. This is in accordance the fact that the antioxidative compounds are mostly non volatile ones which appeared in the second period of the immersion.

4.3. Aromatizing effect

The aromatizing effect was studied by frying tests. Potato chips and pork chops were fried under household conditions and sensory measurements were done by human sensory panel. Based on the results of the aroma development 21 days of immersion was chosen for each concentrations.
Potato chips which were fried in flavoured oil showed different aroma than the control even in the case of small concentrations. The sensory panel described the samples fried in small concentration oil as “green-herb” and the two higher concentrations as “ rosemary-like”. It was in accordance with the phenomena of aroma development.
Pork chops which were fried in flavoured oil did not show clear differencies . The samples fried in small concentration oil did not differ significantly from the control. Only the higher concentrations proved be different. 1.5 % concentration was characterized as “ herb-like” and 2.0% concentration was “ rosemary-like” .
As a summary of the trials I could conclude that aromatizing depends on the type of the fried foodstuff. Potato chips have loose tissue structure and the oil could easily diffuse in to the pieces. Pork chops have firm structure and the oil could only partly diffuse in to the tissue. For industrial application it should be important to choose the appropriate flavoured oil for frying a certain food.

4.4 Heat stability

Due to the sufficient aromatizing effect flavoured oils which prepared with 2.0% rosemary and immersed for 21 days were involved into the heat stability investigation.
Heating temperature was 180°C and the heating time is 2 hours and 6 hours. These parameters simulate a shorter and a longer period of frying.
From the results of the analytical measurements the following conclusions can be drawn. Acidity i.e. the amount of the free fatty acids increased slowly comparing to the control. Even 6 hours heating could cause 2.4 acidity number while in the control it was 12. Similar tendency was observable in the case of peroxide number and anisidine number. Results were in accordance with the results of Rancimat test. As it was reported in several scientific reports the rosemary flavoured sunflower oil showed better heat stability than the unflavoured one.
Analyzing the phytosterol derivatives I got the same result. The phytosterol derivatives in the heated flavoured oil were approximately one third of the amount what was measured in the control both in the case of 2 hours and 6 hours treatment.
Finally the total amount of polar compounds in the flavoured oil was less then the allowed 25 %. Consequently the immersed oil is suitable for frying longer time.

5. SUMMARY

The objective of my work was to study the aroma character and the most important chemical properties of the flavoured sunflower oil made by immersion technology.
The investigations aimed to answer the following questions:

1. How develops the aroma profile of the rosemary flavoured sunflower oil as a function of the amount of the immersed rosemary and the length of immersion time?
2. What is the oxidative stability of the flavoured oil ?
3. What aromatizing effects have the flavoured oils ?
4. What is the heat stability of the flavoured oil ?

A four-part investigation was organized in order to answer the above questions.

1. Dried, cut rosemary leaves were immersed in sunflower oil for a four weeks period by 0.5%, 1.0%, 1.5% and 2.0 %. Aroma development was detected instrumentally by electronic nose chemosensor array, headspace gas chromatography (SPME GC/MS) and by human organoleptic investigations. These letter were completed by textual commentaries too.
   From the results I could state that the aroma modification starts at the very beginning of the immersion regardless the amount of the immersed rosemary. Aroma became stronger during the first two weeks of dipping and caused “fresh”, “herb-like” character in the oil. This is week at the b0.5% samples, medium at the 1.0% samples and favourable at the 1.5% and 2.0% samples. Measurements by gas chromatography showed that during this period the main volatile compounds of the rosemary appeared in the oil. The concentration of these correlated with the amount of the immersed rosemary. Aroma suffered further modifications for the end of the second week especially at the higher concentrations. For the third week of immersion bitter and

typical rosemary aroma appeared. Gas chromatography showed slight increase in the amount of volatile compounds in the oil. For the end of immersion period further modifications happened. The human sensory panel observed an "undesirable" type aroma at the high concentration samples. I could conclude that for the end of immersion the diffusion of non volatile compounds became dominant.

2.The oxidative stability of the rosemary immersed sunflower oil was detected by the Rancimat test. I established that except the 0.5% sample the oxidative stability of the flavoured oils increased comparing to the control. Higher increase were observable in the case of 1.5% and 2.0% samples after two weeks of immersion. The results were in accordance with the measurements of the cinnamic acid derivatives. These accumulated in the oil mainly at the last period of immersion.

3. There were frying test performed to establish the aromatization effect of the oil. Rosemary leaves were immersed in the sunflower oil for 21 days with 0.5%, 1.0%, 1.5% and 2.0% ratio. Potato chips and pork chops were fried under household conditions and human sensory investigations were carried out. Samples were compared to the foods which were fried in unflavoured oil. In case of potato chips the sensory panel found the treated samples to be different from the control and the 2.0% samples proved to be the most favourable. In the case of pork chops the differencies were not obvious. Only the 1.5% and 2.0% samples differed from the control significantly and only the 2.0% samples showed the characteristic rosemary aroma. My conclusion was that the 2.0% rosemary and 21 days immersion is suitable to produce oil with good aromatizing effect.

4. Based on the results of the investigations for the aromatization effect only the 2.0% and 21 days immersed samples were involved to the heat stability tests. Samples were heated up to 180°C for 2 hours and 6 hours. Acidy, peroxide number, anisidine number and the total polar compounds were measured as basic analytical characteristics. Sensory changes were detected by electronic nose system. Of the minor components of the oil, phytosterol derivatives were analyzed. Measurements proved that the acidy, peroxide number anisidine number and the amount of the total polar fraction is less in the flavoured oil than in the unflavoured control. Phytosterol derivatives changed by the same tendency. The total amount of the phytosterol derivatives were less the 1% in the flavoured samples and more than 2% in the control. I could conclude that immersion of 2 % rosemary for 21 days can sufficiently increase the heat stability of the sunflower oil.
Based on the four investigations the followings are stated :

1. Active materials (aroma compounds and antioxidative components) can sufficiently transfer to the sunflower oil by the immersion of dried rosemary leaves. For this purpose the amount of the rosemary must be 2 % and the immersion period is maximum 3 weeks.
2. Methods which were applied during the work proved to be appropriate in answering the questions of the research.

It can be suggested for industrial application the 2% oil with at most two weeks immersion for salad oil and the same concentration with three weeks immersion as aromatized frying oil.

5. NEW SCIENTIFIC ACHIEVMENTS (THESIS)

There were four objectives stated in the present work. Investigations were organized such a way that the objectives should be achieved. The next new results were obtained.

1. For the aroma development of the rosemary –immersed sunflower oil I stated :
   Develpment of aroma depends on the **immersion time** by the following: the dominant aroma compounds appear in the oilafter one day of immersion and causes fresh character in the smell but not observable in taste. There are further modifications in the aroma: within the first two weeks a fresh, primary character appears and during the next two weeks secondary characters are observable.
   Aroma depends on the **concentration of immersed rosemary too.** In the first period of immersion the intensity is in correlation with the concentration and at the end of the immersion two clusters are formed. The two small concentrations (§.5% and 1.0%) belong to the first and the two higher concentrations (1.5% and 2.0%) belong to the other. These letter show undesirable character too. I established that for favourable sensory properties can be achieved at most three weeks of immersion under the circumstances of the present experiment.

2. For the **oxidative stability** of the immersed oils I established that only the 1.5% and 2.0% samples can substantially increase the oxidative stability. For this effect at least two weeks of immersion is needed. I proved that the oxidative stability can increase twofold comparing to the control. Based on the measurements I calculated a two variable exponential equation of the response surface. I proved additionally that the cinnamic acid derivatives which are considered as the antioxidative agents of rosemary appears in the oil after two weeks of immersion and in the case of 1.5% and 2.0% samples.

3. For the **aromatizing effect** I established that only the 2.0% immersed rosemary is suitable to produce flavoured sunflower oil which can transfer the rosemary aroma in the foods during frying. Smaller concentrations can cause only moderate effects.

4. For the **heat stability** I established that the heat stability of the sunflower oil can increase with 2.0% rosemary and three weeks of immersion. Total amount of polar compounds are less then in the control after 2 hours and 6 hours heating and does not reach the maximally allowed level. During heating the acidity, the peroxide number and the anisidine number increases less then in the control. The phytosterol derivatives formed in fewer amounts in the flavoured oil then in the unflavoured control. The modification of the
   sensory properties are more moderate in the flavoured oil comparing to the control.

5. As a summary I established that by immersion it is possible to produce flavoured oil which has specially utilizable aroma character and has better oxidative and heat stability then the unflavoured sunflower oil.

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