Baltic herring (Clupea harengus membras) protein isolate produced using the pH-shift process and its application in food models

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ABSTRACT

In this study, protein isolate was prepared from Baltic herring (Clupea harengus membras) using alkaline pH-shift process. The aim of this research was to characterize the protein isolate and to study its potential in food models. A special focus was placed on characterization of odour profile and volatile compounds contributing to the odour profile of the protein isolate using gas chromatography - olfactometry. 2,3-Pentanedione, hexanal, 4(Z)-heptenal, 2,4(E,E)-nonadienal, and three compounds tentatively identified as 1,5(E)-octadien-3-ol, 1,5(Z)-octadien-3-ol, and 1,5(Z)-octadien-3-one were the most important odour-contributing compounds in the protein isolate (Nasal impact factor 83–100%, intensity 2.6–3.3 on a scale 0–4). Surimi-type gels prepared from the Baltic herring protein isolate had texture properties (hardness and cohesiveness) similar to those of commercial products. Due to the abundancy of dark muscle tissue in Baltic herring, the protein isolate had a significantly lower whiteness (W = 63) compared to the commercial surimi products (W = 80–83). Increasing the solubilisation or precipitation pH did not improve the whiteness, but resulted in significantly softer, less cohesive, and less chewy gels. The findings of this study indicate that alkaline-based pH-shift processing is a potential way to increase the food application of Baltic herring.

1. Introduction

Fish and fish products are excellent sources of nutrients and play a crucial role in protein security. However, this requires a more balanced use of the available fish stocks, as many of them are already over-exploited (FAO Commission on Genetic Resources for Food and Agriculture, 2019), whereas, for example, small pelagic fish are under-utilized as food. Baltic herring (Clupea harengus membras) is the most significant fish caught in Finland, both in quantity and commercial value, although its consumption has decreased ten-fold in the past two decades (Natural Resources Institute Finland, 2020). Baltic herring is a small fish, typically only 15–20 cm long. Juvenile fish are often too small for automated filleting machineries, which decreases its commercial value. In addition, the consumer demand for Baltic herring is low compared to, for instance, Baltic salmon (Pihlajamäki, Asikainen, Ignatius, Haapasaaari, & Tuomisto, 2019). Only approximately 3% of the catch goes into domestic food use, while the majority of the catch is destined for feed for fur animals and farmed fish (Natural Resources Institute Finland, 2021).

Production of protein isolates using the pH-shift method has been suggested as a potential way to provide added value for under-utilized fish species, such as small pelagic fishes. The process can be applied for both whole fish and by-products (Abdollahi & Undeland, 2019; Chomnawang & Yongswatdigul, 2013; Marmon & Undeland, 2010; Surasani, 2018). In the pH shift, fish proteins are collected by first solubilising the proteins at high > 10.8 (alkaline process) or low < 3.0 (acidic process) pH, and then precipitating at the isoelectric point of the proteins. The alkaline process has been shown to be more favourable in terms of yield (Abdollahi & Undeland, 2019), lipid removal (Zhong et al., 2016), and gel forming ability (Phetsang, Panpipat, Undeland, et al., 2021). In addition, most studies have reported the alkaline process to result in protein isolates with a lower degree of oxidation (Kristinsson & Hultin, 2004; Zhong et al., 2016), although contradictory findings have been reported (Abdollahi, Olofsson, Zhang, Alminger, & Undeland, 2019).
During the pH-shift extraction of fish proteins, majority of the lipids are removed. The removal of lipids is desired due to their negative effect on the stability and sensory properties. Most odour compounds in fish are lipid derived, originating from polyunsaturated fatty acids due to the activity of lipoxygenase or autoxidation of lipids (Hsieh & Kinsella, 1989; Lindsay, 1990), and the extent of lipid removal is likely to have an impact on the fishy odour and flavour.

Since the pH shift does not involve heating, or hydrolysis of the protein to a large extent, the extracted proteins retain the functional properties of native proteins, such as gelation. The process has been considered as an alternative for conventional production of surimi, in which the fish muscle is first mechanically separated and then undergoes a washing process, leading to loss of more soluble proteins (Nolsøe & Undeland, 2009). However, the high content of dark muscle in small pelagic fish, such as Baltic herring, poses a challenge on the colour and texture of potential applications. Dark muscled fish usually contain more sarcoplasmic proteins and lipids, which may interfere with gelation properties (Abdollahi & Undeland, 2019). The high content of heme pigments may impart a darker colour in the extracted protein isolate. Potential applications of fish protein isolate produced by the pH shift include use as an emulsifier (Nolsøe & Undeland, 2009), as a functional

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**Table 1** Composition of fish ball mixtures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein isolate (w-%)</th>
<th>Baltic herring mince (w-%)</th>
<th>Canola oil (w-%)</th>
<th>Dry ingredients (w-%)</th>
<th>Water (w-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB0</td>
<td>0</td>
<td>55</td>
<td>10</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>FB5</td>
<td>5</td>
<td>55</td>
<td>10</td>
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<td>23</td>
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</tr>
<tr>
<td>FB28</td>
<td>28</td>
<td>52</td>
<td>9</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>FB50</td>
<td>50</td>
<td>38</td>
<td>7</td>
<td>5</td>
<td>0</td>
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</tbody>
</table>

---

**Table 2** Composition of the raw material (gutted Baltic herring) and protein isolate.

<table>
<thead>
<tr>
<th></th>
<th>Protein (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Lipids (%)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Moisture (%)&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gutted &amp; beheaded Baltic herring</td>
<td>14.7 ± 0.8</td>
<td>6.1 ± 0.1</td>
<td>80.2 ± 0.0</td>
</tr>
<tr>
<td>Protein isolate (incl. 4% of cryoprotectants)</td>
<td>9.2 ± 0.3</td>
<td>3.7 ± 0.0</td>
<td>85.3 ± 0.0</td>
</tr>
</tbody>
</table>

<sup>1</sup> Measured as the total nitrogen content and calculated using nitrogen conversion factor 6.25 (Latimer, 2016) (n = 2).
<sup>2</sup>Measured gravimetrically after chloroform-methanol extraction (Folch et al., 1957) (n = 3).
<sup>3</sup>Measured gravimetrically after drying (Association of Official Analytical Chemists, 2005) (n = 2).
Table 3
Significant odour compounds (NIF > 33%) in Baltic herring protein isolate (PI) and minced Baltic herring (RM) ($n = 6 \times 2$). Rows written in bold indicate that the NIFs and/or intensities of the compound differ statistically significantly ($p < 0.05$) between RM and PI.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Identification</th>
<th>RI SPB-624</th>
<th>RI DB-WAX</th>
<th>Odour description (PI)</th>
<th>Odour description (RM)</th>
<th>NIF (%)</th>
<th>NIF (%) PI</th>
<th>Intensity RM</th>
<th>Intensity PI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>unknown</td>
<td></td>
<td>&lt;600</td>
<td></td>
<td>solvent, pungent</td>
<td>rotten egg, musty</td>
<td>42%</td>
<td>42%</td>
<td>1.2</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>unknown</td>
<td></td>
<td>&lt;600</td>
<td></td>
<td>musty, fish, cheese,</td>
<td>–</td>
<td>50%</td>
<td>25%</td>
<td>1.7</td>
<td>1.0*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>propanol</td>
<td>std, ms, O, RI</td>
<td>&lt;600</td>
<td>797</td>
<td>chemical, pungent,</td>
<td>solvent, chemical, glue, pungent</td>
<td>67%</td>
<td>92%</td>
<td>1.4</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2-methylpropanol</td>
<td>std, ms, O, RI</td>
<td>&lt;600</td>
<td>816</td>
<td>chocolate, cognac,</td>
<td>–</td>
<td>83%</td>
<td>8%*</td>
<td>2.2</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2,3-butanedione</td>
<td>std, ms, O, RI</td>
<td>636</td>
<td>978</td>
<td>caramel, butter, sweet,</td>
<td>caramel, sweet, butter, popcorn</td>
<td>92%</td>
<td>92%</td>
<td>2.9</td>
<td>2.3</td>
<td>1, 3, 4</td>
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<tr>
<td>6</td>
<td>2-methyl-1-propanol</td>
<td>O, RI</td>
<td>681</td>
<td>n.d.</td>
<td>solvent, sweet, pungent,</td>
<td>chemical, pungent, solvent, cognac</td>
<td>67%</td>
<td>58%</td>
<td>1.6</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3-methylbutanal</td>
<td>std, ms, O, RI</td>
<td>699</td>
<td>920</td>
<td>musty, cacao, chocolate,</td>
<td>–</td>
<td>92%</td>
<td>50%</td>
<td>3.2</td>
<td>1.5*</td>
<td>1, 2, 3</td>
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<tr>
<td>8</td>
<td>2-methylbutanal</td>
<td>std, ms, O, RI</td>
<td>707</td>
<td>n.d.</td>
<td>sweet, chemical, stale, chocolate</td>
<td>chemical, green, mushroom, pungent butter, caramel, popcorn, cream</td>
<td>75%</td>
<td>83%</td>
<td>2.3</td>
<td>2.1</td>
<td></td>
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<tr>
<td>9</td>
<td>1-penten-3-ol</td>
<td>std, ms, O, RI</td>
<td>735</td>
<td>n.d.</td>
<td>Pungent, solvent, chemical, green,</td>
<td>92%</td>
<td>92%</td>
<td>3.1</td>
<td>3.3</td>
<td>1, 2, 4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2,3-pentanediol</td>
<td>std, ms, O, RI</td>
<td>744</td>
<td>1058</td>
<td>butter, caramel, sweet,</td>
<td>83%</td>
<td>75%</td>
<td>2.1</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>unknown</td>
<td></td>
<td>779</td>
<td></td>
<td>sweet, fat, solvent, chemical, wax, plastic</td>
<td>83%</td>
<td>75%</td>
<td>2.1</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2(Z)-penten-1-ol hexanal</td>
<td>std, ms, O, RI</td>
<td>830</td>
<td>n.d.</td>
<td>Solvent, grass, green, stale, grass</td>
<td>25%</td>
<td>50%</td>
<td>1.0</td>
<td>1.0</td>
<td>2, 5</td>
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<td>13</td>
<td>2-methylpropanoic acid</td>
<td>O, RI</td>
<td>850</td>
<td>1085</td>
<td></td>
<td>grass, green,</td>
<td>100%</td>
<td>100%</td>
<td>2.9</td>
<td>3.3</td>
<td>3, 6</td>
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<tr>
<td>14</td>
<td>ethyl 3-methylbutanoate</td>
<td>std, ms, O, RI</td>
<td>881</td>
<td>1071</td>
<td>fresh, citrus, fruity, sweet</td>
<td>17%</td>
<td>42%</td>
<td>1.5</td>
<td>2.0</td>
<td>2, 7</td>
<td></td>
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<tr>
<td>15</td>
<td>4(Z)-heptenal</td>
<td>std, ms, O, RI</td>
<td>958</td>
<td>1242</td>
<td>fish, fish oil, rancid, stale,</td>
<td>42%</td>
<td>42%</td>
<td>1.6</td>
<td>1.4</td>
<td></td>
<td></td>
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<tr>
<td>16</td>
<td>unknown</td>
<td></td>
<td>985</td>
<td></td>
<td>–</td>
<td>stale, rancid, spoiled fish</td>
<td>92%</td>
<td>100%</td>
<td>3.3</td>
<td>3.5</td>
<td>1, 4, 6</td>
</tr>
<tr>
<td>17</td>
<td>unknown</td>
<td></td>
<td>992</td>
<td></td>
<td>–</td>
<td>stale, mushroom, potato, soil</td>
<td>92%</td>
<td>67%</td>
<td>2.5</td>
<td>1.5*</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>unknown</td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>19</td>
<td>1-octen-3-one</td>
<td>O, RI</td>
<td>1004</td>
<td>1303</td>
<td>mushroom, metallic, rancid, mushroom,</td>
<td>mushroom</td>
<td>67%</td>
<td>50%</td>
<td>2.1</td>
<td>1.7</td>
<td>1, 2</td>
</tr>
<tr>
<td>20</td>
<td>1,5(E)-octadien-3-ol</td>
<td>O, RI</td>
<td>1039</td>
<td>1358</td>
<td>mushroom, spicy, rose,</td>
<td>spicy, fatty,</td>
<td>50%</td>
<td>83%</td>
<td>2.3</td>
<td>2.7</td>
<td>1, 2</td>
</tr>
<tr>
<td>21</td>
<td>1,5(Z)-octadien-3-ol</td>
<td>O, RI</td>
<td>1045</td>
<td>1486</td>
<td>mushroom, stale, forest</td>
<td>mushroom, forest, green, pungent forest, raw fish, raw carrot, pelargonium</td>
<td>100%</td>
<td>83%</td>
<td>3.0</td>
<td>2.8</td>
<td>1, 2, 4, 8</td>
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<td>22</td>
<td>1,5(Z)-octadien-3-one</td>
<td>O, RI</td>
<td>1055</td>
<td>1377</td>
<td>metal, green, raw fish, rancid,</td>
<td>83%</td>
<td>92%</td>
<td>3.3</td>
<td>3.2</td>
<td>1, 2</td>
<td></td>
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<tr>
<td>23</td>
<td>octanal + 2,4(E,E)-heptadienal</td>
<td>std, ms, O, RI</td>
<td>1073</td>
<td>1291, 1467</td>
<td>citrus, lemon, fresh,</td>
<td>citrus, lemon, fresh,</td>
<td>92%</td>
<td>92%</td>
<td>2.3</td>
<td>2.5</td>
<td>1, 2, 3, 6, 9</td>
</tr>
<tr>
<td>24</td>
<td>unknown</td>
<td></td>
<td>1114</td>
<td></td>
<td>mushroom,</td>
<td>mushroom</td>
<td>33%</td>
<td>25%</td>
<td>1.5</td>
<td>2.0</td>
<td></td>
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<tr>
<td>25</td>
<td>3,5-octadien-2-one (E,E or E,Z)</td>
<td>ms, O, RI</td>
<td>1154</td>
<td>n.d.</td>
<td>plastic, pungent, mushroom,</td>
<td>mushroom,</td>
<td>42%</td>
<td>58%</td>
<td>1.8</td>
<td>1.3</td>
<td>1, 3, 9</td>
</tr>
<tr>
<td>26</td>
<td>3,5-octadien-2-one (E,E or E,Z)</td>
<td>ms, O, RI</td>
<td>1172</td>
<td>n.d.</td>
<td>mushroom, soil, forest</td>
<td>mushroom,</td>
<td>33%</td>
<td>42%</td>
<td>1.5</td>
<td>1.2</td>
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</tr>
<tr>
<td>27</td>
<td>unknown</td>
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<td>1187</td>
<td></td>
<td>mushroom, sweet</td>
<td>mushroom, fresh, sweet</td>
<td>42%</td>
<td>75%</td>
<td>1.8</td>
<td>1.1*</td>
<td></td>
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<tr>
<td>28</td>
<td>unknown</td>
<td></td>
<td>1197</td>
<td></td>
<td>–</td>
<td>fresh, citrus, sweet, chemical</td>
<td>17%</td>
<td>75%*</td>
<td>1.0</td>
<td>1.7*</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>unknown</td>
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<td>1246</td>
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<td>mushroom, hay,</td>
<td>mushroom</td>
<td>58%</td>
<td>25%</td>
<td>1.4</td>
<td>1.7</td>
<td></td>
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<tr>
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<td></td>
<td>cucumber, green,</td>
<td>mushroom,</td>
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<td>42%</td>
<td>2.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>2,4(E,E)-nonadienal</td>
<td>std, O, RI</td>
<td>1296</td>
<td>1680</td>
<td>cucumber, green, flower field, vegetable</td>
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<td>83%</td>
<td>83%</td>
<td>2.6</td>
<td>2.6</td>
<td>1, 2, 4</td>
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<td>32</td>
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<td>1368</td>
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<td>67%</td>
<td>2.3</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>unknown</td>
<td></td>
<td>1412</td>
<td></td>
<td>–</td>
<td>sweet, pungent,</td>
<td>50%</td>
<td>33%</td>
<td>1.3</td>
<td>2.0*</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>unknown</td>
<td></td>
<td>1438</td>
<td></td>
<td>–</td>
<td>green, forest, sprig, mushroom</td>
<td>42%</td>
<td>25%</td>
<td>1.8</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>unknown</td>
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<td>1470</td>
<td></td>
<td>soil, fish</td>
<td>sweet, candy, fruit, acidic</td>
<td>67%</td>
<td>50%</td>
<td>2.0</td>
<td>1.7</td>
<td></td>
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<tr>
<td>36</td>
<td>unknown</td>
<td></td>
<td>1534</td>
<td></td>
<td>pungent, acidic</td>
<td>plant, green, musty</td>
<td>50%</td>
<td>25%</td>
<td>1.8</td>
<td>1.3</td>
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(continued on next page)
Table 3 (continued)

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Identification</th>
<th>RI SPB-624</th>
<th>RI DB-WAX</th>
<th>Odour description (PI)</th>
<th>Odour description (RM)</th>
<th>NIF (%) PI</th>
<th>NIF (%) RM</th>
<th>Intensity PI</th>
<th>Intensity RM</th>
<th>Reference</th>
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<td>unknown</td>
<td>1581</td>
<td></td>
<td></td>
<td>mint, old salad, wholegrain, something, forest</td>
<td>egg, spoiled, sawdust</td>
<td>33%</td>
<td>33%</td>
<td>1.5</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

*An asterisk after the NIF or intensity of PI, indicates that it differs statistically significantly (p < 0.05) from RM.

n.d. = odour not detected.


3Aitta et al. (2021).

4An et al. (2020).


7Martínez-Arellano, Flores, & Toldrá (2016).

8Serot, Regost, and Arzel (2002).

9Ahonen et al. (2022).

Food ingredient to increase the nutritional value (Shaviklo, Thorkelsson, Sveinsdottir, & Rafipour, 2011), or as the main ingredient in products such as fish balls (Shaviklo, Arason, Thorkelsson, Sveinsdottir, & Martinsdottir, 2010) and surimi products (Pires, Batista, Fradinho, & Costa, 2009) to replace surimi or minced fish.


The aim of this work was to investigate the basic composition as well as odour properties and individual odour-contributing compounds of Baltic herring protein isolate produced using pH-shift processing and to study the effects of incorporating the protein isolate in two different types of food models: fish balls and surimi-type gels. The odour properties were analysed using gas chromatography-olfactometry (GC-O) studies on individual compounds responsible for the total odour, and only a few studies on product applications (Pires, Batista, Fradinho, & Costa, 2009; Shaviklo, Arason, Thorkelsson, Sveinsdottir, & Martinsdottir, 2010; Shaviklo, Thorkelsson, Sveinsdottir, & Rafipour, 2011).

2. Materials and methods

2.1. Fish raw materials and chemicals

The raw material was gutted and beheaded Baltic herring (approximately 60% of whole fish weight) from Martin Kala Oy (Turku, Finland). The fish used for the preparation of protein isolate (PI) for studying the odour and odour-active compounds was caught in December 2019, and the PI used for other analyses was prepared from fish caught in March 2019.

Two commercial surimi products (CSs), CS1 (imitation crab stick, “Ravunmakukainen kalapuikko”) and CS2 (imitation crab stick, “Surimi rapupuikko”), were obtained from Ayräpiirtä Port Sales Oy (Helsinki, Finland) and AK Seafood (Vantaa, Finland), respectively. Commercial fish ball reference sample, CFB (frozen fish ball, “Särkisen kalapuukori”), was manufactured by Apetit Ruoka Oy (Helsinki, Finland).

Sorbitol and fructose (Vinoferm® by Brouland, Belgium) were obtained from Lappo Oy (Piikkiö, Finland). Methanol for the solvent extraction was purchased from Honeywell Riedel-de Haën Co. (Seelze, Germany) and chloroform from Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA). The volatile standards, ethyl isovalerate, 3-methylbutanal, 2,3-butanedione, nonanal, octanal, cis-2-penten-1-ol, 2-methylpropanal, 1-octen-3-one, 2,3-pentanedione, trans,trans-2,4-nonadienal, cis-4-heptenal, and hexanal were acquired from Sigma-Aldrich (St. Louis, MO, USA). 1-penten-3-ol was purchased from Fluka Chemicals (Neu Ulm, Switzerland). 2-methylbutanal and propanal were from Acros Organics (Geel, Belgium). The volatile standards were diluted in propylene glycol (Amresco, Solon, OH, USA).

2.2. Preparation of protein isolate and food models

2.2.1. Preparation of Baltic herring protein isolate using the pH shift

The preparation of Baltic herring PI using the pH shift and an overview of the whole study is shown in Fig. 1. The degutted and beheaded fish was stored at −80°C and thawed at 4°C overnight before the protein extraction. Defrosted fish were rinsed and homogenised with water (100 g fish + 800 g water) using a professional mixer (Raw Mix 2, Rawmix Oy, Helsinki, Finland). The pH-shift process was performed in a double-jacketed glass reactor (1 L, 100 mm diameter, Lenz Laborglas GmbH & Co. KG, Wertheim, Germany) cooled to 4°C. Stirring (800 rpm) was achieved with a teflon plade impeller, and acid/base addition was conducted using a precision pump (Alaris Asena GH MK III, Becton, Dickinson and Company, Franklin Lakes, USA) at a constant rate of 30–40 mL/h. To solubilise the proteins, 10–15 mL of 1 M NaOH was added for approx. 15–25 min until the target pH 11.2 ±0.05, chosen based on previous literature (Marmon & Undeland, 2010) and a pre-test on yield (data not shown), was reached. The homogenate was then centrifuged (Centrifuge 6/720R, MSE, Nuaille, France) at 4,000g for 20 min at 5°C. The supernatant containing the solubilised muscle proteins was separated from the sediment and lipid emulsion layer by decanting and filtering through a 4-fold cotton cheesecloth (Decola, Jyväskylä, Finland) and a metal sieve. Proteins were precipitated by adding 12–17 mL of 1 M HCl within approx. 20–30 min, until pH 5.4 ±0.05 was reached, after which the mixing was continued for further 5–10 min. The mixture was centrifuged again (4,000g, 20 min, 5°C), and the precipitate was collected by removing the solid lipid emulsion layer and the supernatant by decanting. The sediment containing the precipitated proteins was collected as PI, and cryoprotectants were added to the PI according to intended use. For surimi-type gels, a total amount of 8%
(w/w) of cryoprotectants (4% fructose and 4% sorbitol) was used, whereas for fish balls, a lower amount of 4% (w/w) (2% fructose and 2% sorbitol) was used. The pH of the isolate was adjusted to 7.0 using 4 M NaOH while mixing manually, after which the neutralized PI was frozen at $-80^\circ$C.

In addition, modifications to the process were done according to Marmon, Krona, Langton, and Undeland (2012) to increase the whiteness value of the PI. The first modification included precipitation of the proteins at pH 6.5, instead of 5.4, and the second modification was solubilisation at pH 11.5, instead of 11.2. Other stages of these processes were conducted as described above.

### 2.2.2. Preparation of food models (surimi-type gels and fish balls)

The PI with a total content of 8% cryoprotectants (4% fructose and 4% sorbitol) was used to prepare the surimi-type gels, whereas the PI with 4% of cryoprotectants (2% fructose and 2% sorbitol) was used as an ingredient in fish balls. For the surimi-type gels, the PI (89.5% of total mixture) was mixed with 6.0% of potato starch, 3.0% of egg white powder (Munax Oy Laitila, Finland), and 1.5% of table salt. Titanium dioxide (TiO$_2$, Oy R. Österlund Ab, Helsinki, Finland) was used as a whitening agent in a second trial at a concentration of 0.5%. However, after this study was conducted, TiO$_2$ as a food additive was banned in the EU (News, 2021), and therefore results related to the whiteness of surimi-type gel with TiO$_2$ are only briefly discussed.

Ingredients of the surimi-type gels were mixed for 3 × 10 seconds at full speed with Bamix® Mono (ESGE Ltd, Mettlen, Switzerland) at room temperature. The mass was cooked in 50 mL centrifuge tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany) immersed in a water bath at 90 °C.

Fig. 2. Frequencies of selected odour attributes in Baltic herring and protein isolate (PI) (A) and rated intensities (B) in the check-all-that-apply test, evaluated by the GC-O panelists (n = 6 × 2). An asterisk indicates a statistically significant difference (p < 0.05) between Baltic herring and PI.
**Fig. 3.** The total peak areas of identified volatile compounds, propanal, 1-penten-3-ol, 2,3-pentanedione, hexanal, 4(Z)-heptenal, octanal, hexanal, 2,4(E,Z)-heptadienal, and 3,5-octadien-2-one (E,E or E,Z) in surimi-type gels prepared from protein isolate (SPI, solubilisation pH 11.2, precipitation pH 5.4), protein isolate produced by precipitation at pH 6.5 (SPI6.5, solubilisation pH 11.2), or protein isolate produced by solubilisation at pH 11.5 (SPI11.5, precipitation pH 5.4). Different letters indicate a statistically significant (p < 0.05) difference between samples (n = 3).

for 40 min. The surimi-type gels are later referred to as SPI (surimi-type gel from PI), SPI6.5 (surimi-type gel from PI produced by precipitation at pH 6.5 instead of 5.4), and SPI11.5 (surimi-type gel from PI produced by solubilisation at pH 11.5).

To prepare the fish balls, different amounts of PI (Table 1) were combined with a flavour and oat mixture by Kalaneuvos Oy (Sastamala, Finland), canola oil (Bunge Finland Oy, Raisio, Finland) and minced Baltic herring fillets (Martin Kala Oy, Turku, Finland). Freshly prepared fillets were brought to the laboratory on ice and minced using a food processor (Kenwood Limited, Havant, United Kingdom). Mixing of ingredients was conducted manually. The amount of minced fish, oil and dry ingredients were kept at constant weight for samples including 0–15% PI. The amount of added water was reduced in all the fish ball mass samples in the sample containing 28 and 50% of the PI. Compared to FB0, the total moisture of the samples was reduced by 76 °C (±2 °C).

The cooked fish balls and surimi-type gels used for TPA and volatile analysis were stored at 4 °C overnight after cooking, and their temperature was allowed to reach room temperature before TPA measurement. For other analyses the samples were stored at –80 °C prior to analysis.

### 2.3. Determination of protein, lipid and moisture contents

The protein contents of the raw material and PI were analysed using the Kjeldahl method by the AOAC International (Latimer, 2016). Each sample was measured in duplicate, and the protein contents were determined using the nitrogen conversion factor of 6.25.

The lipid content of the raw material and PI was analysed by using a modified Folch extraction method (Folch, Lees, & Stanley, 1957). In the first phase separation, chloroform, methanol and 0.88% potassium chloride were added in a ratio of 32:16:15 (v/v/v). The lower phase containing lipids was further washed with KCl:MeOH (1:1) and evaporated in a rotary evaporator. Both samples were analysed in triplicate.

The moisture contents of homogenised Baltic herring, PI (2% sorbitol and 2% fructose) and fish balls with 0, 15 and 50% PI added were determined by drying the samples to a constant weight at 105 °C in an oven (Memmert Loading Modell 100–800, Germany) as described by AOAC (Association of Official Analytical Chemists, 2005). Each sample was analysed in duplicate.

### 2.4. Analysis of odour-active compounds and odour profile of Baltic herring protein isolate

#### 2.4.1. Panelists and training

Six voluntary assessors (four women, two men, age 24–38) took part in the GC-O and odour profiling. Informed consent to participate was obtained from the assessors prior to the first session, after providing...
them with information about the procedures, samples, and their right to withdraw from the study at any point. All panelists (staff and students of the Food Sciences unit in the University of Turku, Finland), recruited to this study were required to have extensive training in sensory science and all had regularly participated in sensory studies. Five out of six panelists had prior experience with GC-O. The panelists were further trained to describe and evaluate the intensity of odour compounds of Baltic herring in two sessions. First session included training with GC-O using minced Baltic herring as a sample, and panelists were asked to describe the nature and intensity of the odorants. The second session was a group training including discussion about the first training, and all had regularly participated in sensory studies. Five out of six panelists had prior experience with GC-O. The panelists were further trained to describe and evaluate the intensity of odour compounds of Baltic herring in two sessions. First session included training with GC-O using minced Baltic herring as a sample, and panelists were asked to describe the nature and intensity of the odorants. The second session was a group training including discussion about the first training, and all had regularly participated in sensory studies.

2.4.2. Gas Chromatography-Olfactometry

Odour-active compounds in Baltic herring PI were analysed using GC-O and compared to the raw material (gutted and beheaded Baltic herring). Detection frequency (DF) and direct intensity (DI) methods were used. The intensity was rated on the scale of 0–4, as in the training sessions. The raw material was minced using a food processor with a meat grinder attachment (Kenwood Limited, Havant, United Kingdom).

Raw material and PI (containing 2% fructose and 2% sorbitol) were portioned and stored in zip-lock bags in –80 °C until analysis. Volatiles were extracted by headspace solid-phase microextraction (HS-SPME), using a Divinylbenzene/Carboxen/Polydimethylsiloxane fiber (DVB/ CAR/PDMS, 2 cm, 50/30 μm film thickness, Supelco Inc., Bellefonte, Pennsylvania, USA). Ten grams (±0.1 g) of PI or raw material were weighed into a 50 mL (total volume of bottle 90 mL) erlenmeyer flask, which was immersed in a 40 °C water bath for 20 min, prior to 30 min extraction at 40 °C.

GC-O analyses were conducted using an HP 6890 Series GC with a flame ionisation detector (FID) (Hewlett Packard, Palo Alto, California, USA). Column SPB-624 (30 m, 0.25 mm, 1.40 μm; Supelco Inc.) was used for the separation of odour compounds. The oven temperature was 40 °C for 3 min, then increased by a ramps of 8 °C/min until 150 °C and 10 °C/min until 220 °C, which was held for 10 min. The carrier gas was helium and the total flow was 1.4 mL/min. Each sample was analysed twice by each panelist using the SPB-624 column. In addition, DB-WAX (60 m, 0.25 mm, 0.25 μm; Agilent Technologies, Santa Clara, California, USA) was used to help identification and to detect possibly co-eluting odour compounds, and two assessors evaluated the samples twice using this column. With DB-WAX, other parameters remained the same, but the oven programme was: 40 °C (held 3 min) followed by a 7 °C/min increase until 220 °C (hold 10 min). The flow from SPB-624 column was directed to either the olfactory detection port (Gerstel, Linthicum, Maryland, USA), or the FID, but with the DB-WAX column a Y connector.
was used to divide the eluent between the detection port and FID. The assessors were given a button connected to a microphone. They were requested to report the duration of the odour by pressing the button and describe the odour and its intensity. Recorded audio files were processed using Audacity 3.0.2 (The Audacity Team). Odorants detected on at least 4 out of 12 sessions (Nasal Impact Factor, NIF ≥ 33%) were considered significant. The odorants were identified by comparison to commercial reference compounds and by HS-SPME-GC–MS (as explained in section 2.6.1). The average odour intensity (assessed by 6 panelists in two sessions) for each compound was calculated by only including the intensities given by the assessors who detected the odour (i.e. null values were not included).

2.5. Odour profile

The odour of Baltic herring PI and raw Baltic herring was further evaluated using a CATA (check-all-that-apply) test, and on a scale of 0–4 (1 = very mild, barely noticeable, 2 = mild, 3 = fairly strong, and 4 = strong; 0 = not detected) using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada). Evaluations were carried out in a sensory laboratory following the ISO8589 standard. The order of the samples and attributes was randomized for each panelist, and both samples were evaluated in duplicate. 5 g of PI and minced raw Baltic herring were weighed in 30 mL bottles covered with aluminium foil and coded with randomized three-digit codes. CATA attributes were chosen based on the descriptors of individual odour compounds most commonly reported in the GC-O sessions. The panelists were further requested to rate the intensity of the odour attributes they found to be present in the sample. If an odour attribute was not given an intensity, it was considered to be 0.

2.6. Analysis of volatile compounds by HS-SPME-GC–MS

2.6.1. Identification of odour compounds in Baltic herring and PI

For the identification of odour-active compounds, samples were analysed using TRACE 1310 GC coupled with a ISQ7000 single quadrupole MS and TriPlus RSH autosampler (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Extraction conditions, oven settings and other parameters were the same as reported for each column in section 2.4.2. The MS was operated in electron ionization mode. Transfer line and ion source temperatures were 220 °C and 250 °C, respectively, and spectra were collected in a mass range of 40–300 amu. Data were processed using Chromelon 7.0 (Thermo Scientific™), and the NIST library (version 2.3, National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

Fig. 5. Texture profile analysis attributes, hardness (A), springiness (B), cohesiveness (C), and chewiness (D) of two commercial reference surimi samples (CS1 & CS2) and surimi-type gels produced from Baltic herring protein isolate. SPI was prepared from protein isolate with solubilisation pH 11.2 and precipitation pH 5.4, in SPI6.5 the protein isolate was produced by precipitation at pH 6.5 (solubilisation pH 11.2), and in SPI11.5 the protein isolate was produced by solubilisation at pH 11.5 (precipitation pH 5.4). Different letters indicate a statistically significant (p < 0.05) difference between samples (n = 3).
2.6.2. Analysis of volatile compounds in food models

Volatile compounds in fish balls and surimi-type gels were analysed with HS-SPME-GC-MS. The analysis was conducted similarly as described in section 2.6.1, with slight differences. The surimi-type gels and fish balls were cut to 1 cm$^3$ cubes. A 1 cm DVB/CAR/PDMS fiber (Supelco Inc.) was used. The column used in case of surimi-type gels and fish balls was SPB-624 60 m × 0.25 mm i.d., 1.4 μm film thickness (Supelco Inc.). The oven temperature was held at 40 °C for 6 min, followed by an increase of 5 °C/min to 200 °C, and held there for 10 min.

Fig. 6. Texture profile analysis attributes of fish balls containing Baltic herring and protein isolate (PI); control fish ball with no PI (FB0), fish ball with 5% of mince replaced with PI (FB5), fish ball with 15% of mince replaced (FB15), fish ball with 28% of mince replaced (FB28), and fish ball with 50% of mince replaced (FB50) (n=3). A commercial fish ball (CFB) was included as a reference sample. Hardness (A), springiness (B), cohesiveness (C), and chewiness (D). Different letters indicate a statistically significant ($p<0.05$) difference between samples.

Fig. 7. Colour of commercial surimi preparations (CS1 and CS2) and surimi-type gels prepared from Baltic herring protein isolate. SPI was prepared from protein isolate with solubilisation pH 11.2 and precipitation pH 5.4, in SPI6.5 the protein isolate was produced by precipitation at pH 6.5 (solubilisation pH 11.2), and in SPI11.5 the protein isolate was produced by solubilisation at pH 11.5 (precipitation pH 5.4). W = whiteness, $L^*$=lightness, $a^*$=red-green axis, and $b^*$=yellow-blue axis Different letters above the bars (within the same colour parameter) indicate a statistically significant ($p<0.05$) difference between samples (n = 3).
2.7. Texture profile analysis and colorimetry

Texture profile analysis (TPA) was conducted using a QTS25 texture analyser (CNS Farnell Company, Borehamwood, U.K.), and measurements were carried out at room temperature. The samples were cut to 1.5 × 1.5 × 1.5 cm pieces and measured in triplicate. A cylindrical probe with a 3.8 cm diameter was used to penetrate the sample at the rate of 60 mm/min to a total deformation of 50% of the sample length. The compression cycle was repeated twice for each sample. The measured attributes were hardness, cohesiveness, springiness and chewiness. The prepared surimi-type gels and fish balls were compared to commercial reference samples (CS1, CS2, and CFB).

The whiteness of CSs and the surimi-type gels prepared with the PI were determined by an Eoptis CLM-194 colorimeter (EOPTIS SRL, Trento, Italy). The L*, a*, and b*, representing the black to white, red to green and yellow to blue axes, respectively, were used to determine whiteness (W) values by using the following formula (Park, 2005):

\[ W = 100 - \left( \frac{100 - L^*}{2.44} \right)^2 + a^* + b^* \]

2.8. Statistical analysis

Statistical differences between samples were determined by one-way analysis of variance and Tukey’s test, except for the CAT and GC-O detection frequency data, for which McNemar’s test was used. Statistical comparisons were done using IBM SPSS Statistics 25 (Armonk, New York, United States). The differences were considered statistically significant if \( p < 0.05 \).

3. Results and discussion

3.1. Characterisation of Baltic herring protein isolate

3.1.1. Composition

The mass yield of the isolation process was approximately 82%. Protein, lipid and moisture contents of gutted and beheaded Baltic herring, and PI including 4% of cryoprotectants are presented in Table 2. Recently, Nisov et al. (2020) reported similar protein contents of 14.2 and 14.4%, lipid contents of 2.2 and 2.3%, and moisture contents of 80.3 and 81.8%, respectively, for whole and gutted Baltic herring.

In a previous study on pH-shift processing of herring, the protein content of an isolate produced with alkaline pH shift was 17.8% of fresh weight containing 78% water, and the lipid content was 3.9% (Marmon & Undeland, 2010). Nisov et al. (2021) reported a protein content of 78% (dry matter basis) for Baltic herring protein isolate produced using alkaline pH shift. Here the protein contents were 74% and 85% for the raw material and PI (cryoprotectants excluded) on dry matter basis, respectively.

During processing 37% of the lipids present in the raw materials were removed. Alkaline pH shift has been reported to have better lipid removal efficiency compared to the acid-aided process. Marmon and Undeland (2010) achieved approx. 50% lipid removal from the herring isolate using alkaline pH shift, whereas the acid-aided process removed 38%. Higher efficiencies of lipid removal have been reported by Kristinsson, Theodore, Demir, and Ingadottir (2006) who observed reductions of 85 and 89% by acid- and alkali-aide processes, respectively, on Atlantic croaker, and by Taskaya, Chen, Beamer, Tou, and Jaczynski (2009), who reported lipid removals of 88–89% and 94–97% from silver carp using the acid- and alkali-aide processes, respectively. Some of the variation in lipid removal efficiencies can likely be explained by different centrifugation speeds (8,000–10,000g used by Kristinsson et al. (2006), Taskaya et al. (2009), and Marmon and Undeland (2010) compared to 4,000g in this study) and different composition between fish species, especially related to the proportions of unsaturated fatty acids and phospholipids (Kakko et al., 2022; Marmon & Undeland, 2010).

3.1.2. Odour-active compounds of Baltic herring and PI

Altogether, 33 compounds with a Nasal Impact Factor (NIF) above 33% were detected in the raw material, whereas 29 were detected in the PI (Table 3). Most of the identified odour compounds were alcohols, aldehydes and ketones derived from lipid oxidation. Hexanal (grass, green) was detected by all panelists in both samples, and 4(Z)-heptenal (fish, rancid, stale, green) had NIF values of 92% and 100% in the raw material and PI, respectively. Both of these aldehydes have been previously reported in Baltic herring (Aro, Tahvonen, Koskinen, & Kallio, 2003; Damerau et al., 2020) as well as in several other fishes (Jonsdottir, Bragadottir, & Olafsdottir, 2007; Phetsang, Panipat, Panya, et al., 2021). 4(Z)-heptenal is produced from 2,6(E,Z)-nonadienial (Josephson & Lindsay, 1987), and has been associated or seen to correlate with fishy odour and flavour (Joasquin, Tolas, Oliveira, Lee, & Lee, 2008; Triqui & Bouchriti, 2003), especially in combination with other aldehydes, such as its precursor 2,6(E,Z)-nonadienal (Venkateshwarlu, Le, Meyer, & Jacobsen, 2004). 2,6(E,Z)-octadienal was however not detected (NIF < 33%) in the present study. In addition to hexanal and 4(Z)-heptenal, 2,3-butanedione (caramel, butter), 2,3-pentanedione (butter, caramel), and odorous region corresponding to octanal and/or 2,4(E,Z)-heptadienal, significantly contributed to the odour of both Baltic herring and PI.

Branched aldehydes, 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal, were detected at high frequencies in the raw material (83, 92, and 67%, respectively), unlike in the PI, where the NIF values of these compounds were 8, 50, and 0%, respectively. These branched aldehydes are products of Strecker degradation and key odour/flavour compounds in several types of food (Smit, Engels, & Smit, 2009).

The authors are not aware of previous GC-O studies on fish protein isolate produced using the pH shift. A recent study, however, compared the odour-active compounds of surimi made of silver carp, Pacific whiting, or Alaska pollock (An, Qian, Alcazar Magana, Xiong, & Qian, 2020). Most potent odour-active compounds in the surimi included hexanal, 4(Z)-heptenal, 2,6(E,Z)-nonadienal, 2,4(E,E)-nonadienal, 2,4(E,E) and E,Z-decadienal, 2,3,4(E,Z,Z)-tridecatrienial, and 4,5(E)-epoxy-2(E)-decenal. These compounds contributed to fishy, green, oily, or metallic odours. In the present study, 4(Z)-heptenal and 2,4(E,E)-nonadienal were also considered as important contributors to odour of both raw Baltic herring and PI, due to their high NIFs and intensities. Zhou, Chong, Ding, Gu, and Liu (2016) studied the effect of different washing processes on odourants in the production of surimi from silver carp (Hypophthalmichthys molitrix) mince. The odour activity values of most of the odor-active compounds were decreased by the washing processes, with the saline and mildly alkaline washing solution being more effective than water. The authors hypothesized that the salt could have helped to release volatiles from proteins and thus facilitate their removal during washing, or removal of volatiles together with some of the proteins.

Several factors may influence the content and release of odour-active volatile compounds in the pH-shift process. During the process, a significant portion of lipids are removed. The extent of lipid removal is considered crucial due to the importance of lipid derived compounds for fishy odour and flavour. In the present study, some of the most prominent changes in odour-active compounds occurred in the branched aldehydes 2- and 3-methylbutanal, and 2-methylpropanal, which are not breakdown products of lipids. These aldehydes were however identified in enzymatically extracted Baltic herring oil (Aitts, Marsol-Vali, Damodaran, & Yang, 2021), indicating that they were co-extracted with the lipids. Furthermore, during the pH-shift process proteins undergo significant conformational changes due to the changes in both pH and ionic strength, both of which affect how proteins bind volatiles (Damodaran & Kinseilla, 1983; Pérez-Juan, Flores, & Toldrá, 2006). Gu et al. (2020) showed that the ability of silver carp proteins to bind volatiles was dependent on both pH and ionic strength. In addition, during the pH shift, there is a significant loss of water-soluble proteins and peptides.
during the precipitation and the following separation step. The lower intensities and detection frequencies observed for 2- and 3-methylbutan- nal and 2-methylpropanal in the present study may be explained by their removal together with either lipids or water-soluble proteins. Further, the release of these volatiles might be reduced due to being bound by the sarcoplasmic or myofibrillar proteins, which had undergone structural and conformational changes during the pH-shift process. 2-Methylbutanal and 2-methylpropanal have been previously shown to be bound to sarcoplasmic and myofibrillar protein homogenates from pork muscle (Pérez-Juán et al., 2006).

3.1.3. Odour profile

The frequencies and intensities of odour attributes in Baltic herring and the PI are presented in Fig. 2. CATA attributes were chosen among the most common GC-O descriptors (Table 3), given by the same panelists (n = 6). The raw material was most often chosen as raw fish-like (92%), fish oil-like (92%), and fatty (83%), whereas PI was most frequently chosen to be fatty (58%), fish oil-like (50%), and sweet (42%). The “raw-fish” attribute was chosen statistically significantly (p = 0.008) less often in the case of the PI, compared to the raw material (25% vs 92%). In addition, “fish oil” attribute was chosen almost statistically significantly (p = 0.063) less often in the case of the PI compared to the raw material.

The Baltic herring PI had statistically significantly lower total intensity of odour (1.9 vs. 2.5) and fish-like odour (2.2 vs. 3.3) compared to the minced fish. The difference in the musty odour intensity (1.1 and 2.0 for the PI and the minced Baltic herring, respectively) was almost statistically significant (p = 0.055). Results were in accordance with a study by Phetsang et al., 2021, where fishy and musty off-odours were significantly lower in pH-shift processed protein isolates, compared to the unashed mince or surimi of hybrid catfish. In contrast, Abdollahi et al., (2015) reported herring protein powder prepared by pH shift and freeze-drying being characterised by a high intensity of dried fish and fish oil odours. In the present study, the PI also showed a relatively intense fatty odour (1.8).

3.2. Volatile compounds in food models

Figs. 3 and 4 contain selected volatile compounds and total summed peak areas of identified volatile compounds in surimi-type gels (SPI samples) and fish balls (FBs), respectively. In case of the surimi-type gels, the total peak area of the identified volatiles was significantly (p < 0.05) higher in SPI11.5 compared to the other samples (Fig. 3), while the SPI16.5 had the lowest total peak area (p < 0.05). Propanal, 1-penten-3-ol, 2,3-pentanedione, and hexanal were among the most prominent volatile compounds in all samples. Most of these compounds are secondary lipid oxidation products derived from n-3 fatty acids (Gomez-Cortes, Sacks, & Brenna, 2015), while hexanal can be produced also from n-6 fatty acids (Frankel, Hu, & Tappel, 1989). These compounds were also significant in the PI in terms of odour, based on the GC-O analysis (Table 3). 1-Penten-3-ol, 2,3-pentanedione, and hexanal followed the same trend with total areas, SPI11.5 having the largest peaks (p < 0.05), followed by SPI and SPI16.5. Some compounds, such as 2,4(E, Z)-heptadienal and 3,5-octadien-2-one, did not differ significantly between SPI, and SPI16.5, but their peak areas were significantly higher in SPI11.5 (p < 0.05).

The slight increase in solubilisation pH from 11.2 (SPI) to 11.5 (SPI11.5) caused a significant increase in the total peak area of identified volatile compounds as well as the abundance of individual volatile products of lipid oxidation. Increasing pH has been shown to increase lipid oxidation in water-oil systems (Kim et al., 2016), which might explain the differences in the oxidation-related volatile contents. On the other hand, SPI16.5 had statistically significantly lower areas of hexanal, 2,3-pentanedione, and 1-penten-3-ol compared to other surimi-type gels, where the PI was precipitated at pH 5.4. This indicates that increasing the precipitation pH to 6.5 induced less lipid oxidation.

Marmon et al., (2012) also showed that the protein isolate precipitated at pH 6.5 was less oxidized compared to precipitation at pH 5.5.

In fish balls, the most influential volatile compounds were related to the flavour mixture added to the fish ball mass, and many of the relevant odour-contributing compounds of the PI were not detected. The compounds originating from the flavour mixture included terpenes, such as β-pinene, α- and β- phellandrene, 3-carene, D-limonene, and o-cymene, which are common in herbs and spices (Kruma et al., 2011; Sonmezdag, Kelebek, & Selli, 2015). The most important compounds related to lipid oxidation or contributing to PI odour are presented in Fig. 4. FB0 had significantly lower (p < 0.05) peak area of 2-ethylfuran, pentanal, heptanal and hexanal compared to FB50. On the contrary, the area of 3- methylbutanal was statistically significantly higher, which is comparable to the GC-O results confirming that the compound is more abundant in raw fish compared to the PI. FB0 contained a higher proportion of minced Baltic herring compared to FB50 (Table 1).

3.3. Texture attributes and colour

Fig. 5 displays the TPA results from two different commercial surimi products and four different surimi-type gels produced from Baltic herring. PI in this study. SPI was within the range of the two commercial surimi products (CSs) in terms of hardness. However, changing the precipitation pH to 6.5 (SPI6.5) or solubilisation pH to 11.5 (SPI11.5) significantly softened the gel. All SPIs had higher springiness values than the two CSs. Springiness describes the rate at which a deformed sample returns to its original shape, thus, the SPIs were significantly more elastic in texture compared to the CSs. Cohesiveness evaluates the strength of the internal bonds in the sample. Based on the results, SPIs with modified pH values (SPI6.5 & SPI11.5) were significantly less cohesive compared to the other samples. The softening of the SPI11.5 may have resulted from an increased degree of protein denaturation, and as a result decreased renaturation after precipitation and neutralization (Abdollahi & Undeland, 2019). In addition, as the isoelectric point of the muscle proteins was not reached, the proteins in the SPI6.5 were likely more charged, holding more water leading to an increased water content. Similarly as with hardness and cohesiveness, SPI6.5 and SPI11.5 had significantly lower chewiness values than SPI. PI was significantly higher in chewiness than CS1 and CS2. Based on the results, Baltic herring PI resulted in a comparable gel with the commercial products in terms of texture properties.

The aim of the PI addition was to evaluate whether it can replace some of the minced fish and improve the textural quality of the fish balls. The textural properties of Baltic herring fish balls were further compared to a commercial fish ball (CFB). The addition of 5–50% of Baltic herring PI affected hardness, springiness and chewiness of the fish ball samples (Fig. 6). The addition of 50% of PI resulted in a harder fish ball, but the difference to other Baltic herring fish balls (FB0, FB5, FB15, FB28 and FB50) was not statistically significant when the commercial fish ball (CFB) was included in the comparison. There were no significant differences in other attributes between FB0 and FB50. The lowest springiness was observed in FB5, while the highest chewiness was observed in FB28. Based on the moisture contents, the addition of 50% of PI increased the water content from 60% to 64% compared to the FB0. The added water content, however, did not cause leaking or softer texture. Based on hardness, chewiness, water leaking during cooking, and the roundness of the fish balls (visual observation), the addition of PI improved the texture of the fish balls. Previously, Shavliko et al., (2010) concluded that fish balls containing 75% haddock mince and 25% haddock protein isolate formed better than the control fish balls (100% mince). However, without conducting a consumer test it is difficult to estimate whether the addition of PI would be desirable from a consumer perspective. Compared to CFB, all Baltic herring fish balls were softer and less chewy, regardless of whether PI was included. The difference might be due to different ingredients used or differences in composition, such as moisture content.
Pelagic fish, including Baltic herring, are abundant in dark muscle tissue, which is rich in lipids and pigments, such as hemoglobin and myoglobin. Darker tissue leads to darker protein mass, whereas the fish species conventionally used in surimi production, such as Alaska pollock (Theragra chalcogramma), have white flesh (Park, 2005). The whiteness values of SPIs were significantly smaller than in the commercial surimi (Fig. 7) due to the different raw materials used. Haemoglobin may decrease whiteness and cause yellow or brown colour to the protein. Abdollahi and Undeland (2019) achieved a slightly lighter herring gel by increasing the solubilisation pH from 11.2 to 11.5 due to more efficient removal of heme pigments. Here, changing the solubilisation pH from 5.4 to 6.5 did not improve the whiteness. In one study, acidic pH shift resulted in an increase in whiteness compared to the alkaline processing of herring (57 vs. 61) (Marmor & Undeland, 2010), but in another study, haemoglobin was removed more efficiently with the alkaline processing, especially when precipitation was conducted at pH 6.5 (Abdollahi, Marmon, Chaijan, & Undeland, 2016).

The addition of titanium dioxide increased the whiteness value significantly, from 63 (SPI) to 74. Titanium dioxide was also used successfully in a previous study as a whitening agent in carp gel (Taskaya, Chen, & Jaczynski, 2010). However, TiO2 was recently banned as a food additive in the EU (News, 2021). Novel methods have been studied to increase whiteness of surimi, such as treatment with ozone water, which increased the whiteness of grass carp surimi (Liu et al., 2021).

4. Conclusions

The pH shift has been suggested as an alternative for conventional production of surimi, in which an extensive washing process of mechanically separated fish mince is conducted. Compared to conventional surimi processing, the pH shift has the benefits of leading to a smaller loss of soluble proteins, higher removal of lipids, and not having the need of mechanical separation of muscle prior to processing (Nolsoe & Undeland, 2009; Sasidharan & Venugopal, 2020). The pH shift may also be favourable in terms of functional properties, such as gelation, and elimination of off-odours (Phetsang et al., 2021). In this study, the alkaline pH-shift processing resulted in protein isolate with reduced total odour and fishy odour intensities compared to Baltic herring mince. Based on NIFs (≥83%) and intensities (≥2.5) of individual odour compounds, 2,3-pentanedione, hexanal, 4(Z)-heptenal, 2,4(E,E)-nonaadienal, and three compounds tentatively identified as 1,5(E)-octadien-3-ol, 1,5(Z)-octadien-3-ol, and 1,5(Z)-octadien-3-one were the most significant contributors to the odour of PI. For Baltic herring, 2,3-butanedione, 3-methylbutanal, 2,3-pentanedione, hexanal, 4(Z)-heptenal, 2,4(E,E)-nonaadienal, and tentatively identified 1,5(Z)-octadien-3-ol, and 1,5(Z)-octadien-3-one were the major volatile compounds contributing to the odour. Differences in the odour of Baltic herring and PI could possibly be attributed not only to partial removal of lipids during the pH-shift processing, but also to altered release of volatiles due to changes in the matrix.

The pH shift was shown to have potential as a protein extraction method for Baltic herring. The produced PI was successfully used as an ingredient in food models (surimi-type gels and fish balls). The surimi-type gels prepared from the protein isolate had similar texture properties to commercial surimi-based imitation products, whereas the addition of PI improved the shape and water holding ability of fish balls. Yet, the colour of the SPI was significantly darker than in the commercial products due to the abundance of dark muscle tissue in Baltic herring. The reduced fishy odour seen for the PI compared to the raw material could have a positive effect on sensory perception of the final products. However, a consumer acceptability study of the developed food models is needed to confirm this.

CRediT authorship contribution statement

Tanja Kakko: Conceptualization, Formal analysis, Investigation, Writing – original draft, Funding – review & editing, Visualization. Elia Aitta: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding – review & editing, Visualization. Oskar Laaksonen: Conceptualization, Writing – original draft, Writing – review & editing, Supervision. Pasi Tolvanen: Resources, Writing – review & editing, Supervision. Lauri Jokela: Investigation, Writing – review & editing. Tapio Salmi: Resources, Writing – review & editing, Supervision. Annelie Damerau: Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration. Baoru Yang: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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