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Estimated intake of benzoic and sorbic acid in Denmark

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Abstract

The monitoring of food additives and recent dietary surveys carried out in Denmark have earlier been used to estimate the intake of sweeteners and nitrite in relation to ADI values. The ubiquitous use of the preservatives benzoic and sorbic acid raises the question of the magnitude of the intake of these preservatives in relation to ADI values, which is explored in this paper. The content of benzoic and sorbic acid in all food groups, where they are allowed, has been monitored in Denmark 17 times from 2001 to 2006 with a total of 1526 samples. Transgressions of maximum limits, illegal use or declaration faults have been found in about 3% of the samples. From repeated investigations on fat based foods (salads and dressings), marmalade and stewed fruit it can be concluded that the amounts used by the industry have been relatively stable through the whole period, although limited data for marmalade show some variation. Most foods in the categories soft drinks, dressing, fat based salads, pickled herrings and marmalade contain benzoic and sorbic acid, and sliced bread contain in some cases also sorbic acid. The median daily intake and intake distribution of benzoic and sorbic acid have been calculated with data from the Danish National Survey of Dietary Habits and Physical Activity (age: 4-75 years) conducted in 2000-04 with 5785 participants. The median intakes of both benzoic acid and sorbic acid are well below the acceptable daily intake (ADI) values of 0-5 mg/kg bw/day and 0-25 mg/kg bw/day for benzoic and sorbic acid respectively. However, the 90th percentile based on the average of the samples with a content of benzoic acid is higher than the ADI value for both men and women with the highest value of 16 mg/kg bw/day for both boys and girls in the age group 4-6 year old.. Based on the average of all the samples the 95th percentile

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4 is over the ADI value for men up to 34 years and for women up to 24 years and the 90th percentile
5 for men up to 18 years and for women up to 10 years. Soft drinks, salads and dressing are the main
6 contributors to the benzoic acid intake. The sorbic acid intake based on the average of all samples is
7 well below the ADI value. However, for the intake based on the average of samples with content
8 the 95th percentile exceeds the ADI . This is caused by the dominating contribution to the intake of
9 sorbic acid from sliced bread, but since only 7 out of 42 samples have added sorbic acid, the
10 calculation based on the average of samples with content will exaggerate the intake. With the built
11 in safety factor of 100 in the ADI values and judging from the literature the high intakes of benzoic
12 acid should not cause any concern for ill effects. However, there must be reason to reconsider the
13 maximum limits especially for benzoic acid in soft drinks, dressing and salads and for sorbic acid in
14 sliced bread.
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26 **Keywords:** benzoic acid, sorbic acid, estimated intake, Denmark, monitoring programme
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31 **Introduction**

32 Benzoic acid (E210), sorbic acid (E200) and their salts are food preservatives with antimicrobial
33 effect allowed in practically all food groups in amounts from 200 mg/l in fruit juice and beer to
34 5000 mg/kg in liquid egg products for benzoic acid and 150 mg/l in aromatized non-alcoholic
35 beverages to *quantum satis* for surface treatment of whey cheese for sorbic acid (Fødevarestyrelsen
36 2008). They also occur in nature in small amounts not exceeding 40 mg/kg on average, but in
37 berries of the *Vaccinium* species (cranberries, cloudberry and bilberry) can be found as much as
38 300-1300 mg benzoic acid/kg (WHO 2000). It is the free acids which have the antimicrobial effect,
39 and that is why benzoic and sorbic acid have the best preservative effect in acid or slightly acid
40 foods. However, the salts are much more water soluble than the acids and are therefore often used
41 as the actual food additives. JECFA has established an ADI of 0–5 mg/kg for benzoic acid and
42 benzoates and 0-25 mg/kg for sorbic acid and sorbates (JECFA 1996).
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54 Concern has been expressed over hypersensitivity to food additives where benzoic acid is a likely
55 candidate (Norges Astma- og Allergiforbund 2008, Fødevarestyrelsen 2004). However, the
56 evidence for hypersensitivity of benzoic acid is sparse, and it is assumed that only 1-2% of children
57 react towards food additives at all (Fødevarestyrelsen 2004). Benzoic acid also came under
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4 suspicion being part of the cocktail of artificial colours and additives causing hyperactive behaviour
5 in children (McCann et al, 2007). It is also known that both substances can cause non-
6 immunological contact reactions (pseudoallergy) in sensitive persons in lower doses than 5 mg/kg
7 bw (WHO 2000).
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13 The Danish monitoring programme for food additives (Leth et al. 2007) also includes benzoic and
14 sorbic acid, which has been part of 17 projects from 2001 to 2006 including all food groups where
15 they are allowed.
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20 With the dietary survey carried out by the National Food Institute from 2000 and onwards (Lyhne et
21 al. 2005) and the data from the monitoring programme it was possible to estimate the median daily
22 intake and intake distribution, the results of which and a risk evaluation are presented in this
23 investigation.
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28 29 **Materials and methods**

30 *Monitoring programme*

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32 In the Danish monitoring programme for food additives benzoic and sorbic acid were included in 17
33 projects from 2001 to 2006 covering all food categories, where they are allowed, from cheese,
34 margarine, fat based spreads and salads to soft drinks. In all 1526 samples were taken by the local
35 authorities all over Denmark from producers, importers, and wholesalers covering all brands which
36 could be found on the Danish market. In the food categories fat containing foods, marmalade and
37 stewed fruit analysis was carried out several times during the period 2001-6, making it possible to
38 show trends in the intake of benzoic and sorbic acid in Denmark.
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46 *Chemical analysis*

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48 Benzoic and sorbic acid were extracted from 5 g homogenized food matrix by shaking for 30 min.
49 with 25 ml of a mixture of oxalic acid, ethanol, 2-propanol and acetonitrile (1 g oxalic acid to 125
50 ml ethanol, 125 ml 2-propanol and 250 ml acetonitrile) after addition of methyl-3-hydroxybenzoic
51 acid as internal standard. After centrifugation at 3000 r/min. in 10 min. and freezing of fat
52 containing samples for 2 h the preservatives were determined by reversed phase HPLC on a C18
53 column, 5 µm, 10 cm, gradient elution with a mixture of buffer solution (5 g ammonium acetate and
54 20 ml acetic acid diluted with water to 1000 ml) and acetonitrile and spectrophotometric detection
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4 at 240 nm. Baseline separation between benzoic acid, sorbic acid, methyl-p-hydroxybenzoate,
5 ethyl-p-hydroxybenzoate and propyl-p-hydroxybenzoate was achieved. All chemicals were
6 analytical grade and the solvents were HPLC grade. The laboratory was accredited to perform the
7 method according to ISO 17025 (ISO 2005).
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13 In every series of samples was included a duplicate divided between two series and a recovery
14 experiment with addition of the preservatives to the original sample in the same amount as judged
15 to be in the sample. From the duplicate determinations over the years from 2001 to 2006 the relative
16 internal reproducibility were calculated to be 1.8% for benzoic acid and 1.6% for sorbic acid. From
17 the recovery experiments over the 5 years the recovery rate was found to be 99.5% for benzoic acid
18 with an absolute standard deviation of 2.7% and 98.8% for sorbic acid with an absolute standard
19 deviation of 4.6%. The results showed that the analytical method had been under control at all
20 times. The detection limit was 5 mg/kg for both benzoic and sorbic acid.
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29 *The Danish National Survey of Dietary Habits and Physical Activity*

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31 The survey was conducted in 2000-2004 on a random sample of Danes aged from 4 to 75 years
32 drawn from the Central Population Register (Lyhne et al, 2005) and covers intake data on 5785
33 individuals. Dietary data was based on recording on seven consecutive days. The questionnaire was
34 designed to cover the whole diet. A combination of food quantification methods (household
35 measures and estimation from photographs of different portion sizes) were used to obtain the best
36 estimate of amounts consumed. Body weight was self reported during a personal interview.
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43 *Estimated intake calculation*

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45 Estimation of intake is done by deterministic calculation within the General Intake Estimation
46 System (GIES) developed for the Danish dietary survey (Andersen et al, 2002). A customized
47 recipe collection was developed by a trained dietician in order to link the available analytical data in
48 its fullest extent with the intake registrations.
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54 For exposure assessment of benzoic and sorbic acid two scenarios were used: Scenario 1, called in
55 this study “average intake”, assuming that the consumers may randomly consume food products
56 that contain or not benzoic and sorbic acid; and scenario 2, called in this study “high intake
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calculation”, assuming that the consumers may always consume food products that contain detectable amounts of these two food preservatives.

Results

In table I is shown the average content of benzoic and sorbic acid in all the samples in 29 foodstuff categories, the average only of samples with a content over the detection limit and the number of samples with content as well as the total number of samples in the foodstuff category.

[Insert table I about here]

Of the total number of samples of 1526 three samples had a content of both benzoic and sorbic acid in foods where addition was not allowed, corresponding to 0.2% of the samples. In 42 samples the sum of benzoic and sorbic acid was higher than allowed corresponding to 2.8% of the samples. In 46 samples a declaration for benzoic acid was missing corresponding to 3.0% of the samples and in 30 samples a declaration for sorbic acid was missing corresponding to 2.0% of the samples. In 13 samples benzoic acid was declared but not found corresponding to 0.9% of the samples and in 15 samples sorbic acid was declared but not found corresponding to 1.0% of the samples.

Fat containing foodstuffs like dressing, mayonnaise, remoulade, bernaise sauce, fat based salads and spreadings have been analysed four times in the period from 2001 to 2006 on 743 samples and the results are given in table II.

[Insert table II about here]

Most of the foods in this category have a content of both benzoic acid (from 63% to 94%) and sorbic acid (from 95% to 100%). However, the use of preservatives in these types of foodstuffs seems more or less constant over the years although with some fluctuations for benzoic acid, since the average content in samples with a content is the same in 2001 as in 2006.

The three investigations of marmalade from 2001-2004 (table III) show from 20% to 22% of marmalade samples with content of benzoic acid and from 45% to 51% with sorbic acid. There are

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5 considerable fluctuations over the years especially for benzoic acid. However, no clear tendency in
6 the development over the years can be seen.
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10 [Insert table III about here]
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13 Also stewed fruit has been investigated three times from 2001 to 2004 (table IV), but only a few
14 products could be found on the market, and no clear tendency in the content of benzoic acid (from
15 20% to 38% with content) and sorbic acid (from 40% to 75% with content) can be found.
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19 [Insert table IV about here]
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22 In figure 1 and 2 is shown the intake distribution for benzoic acid for men and women respectively
23 both calculated with the average content of all samples (average intake calculation) and with the
24 average content of samples with content (high intake calculation). The ADI of 0-5 mg/kg bw/day is
25 given in the figure.
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31 [Insert figure 1 and 2 about here]
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34 Similarly, in figure 3 and 4 is shown the intake distribution for sorbic acid for men and women
35 respectively both calculated with the average content of all samples (average intake calculation) and
36 with the average content of samples with content (high intake calculation). The ADI of 0-25 mg/kg
37 bw/day is given in the figure.
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43 The figures show the intake distribution by 10 age classes and in all sampled subjects.
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47 [Insert figure 3 and 4 about here]
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51 In figure 5 to 8 is shown the contribution of the various food groups to the intake of benzoic and
52 sorbic acid calculated for the whole group of people 4-75 years old with the average of all the
53 samples and with the average of the samples with a content of benzoic or sorbic acid (high intake
54 calculation). Calculations for the individual age groups show the same pattern (data not shown).
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56 Soft drinks are the main contributor to the intake of benzoic acid, where the average intake of soft
57 drinks with carbon dioxide, light is 24 ml/day; with carbon dioxide, sugar sweetened 108 ml/day;
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4 without carbon dioxide, light 71 ml/day and without carbon dioxide, sugar sweetened 136 ml/day
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6 (Leth et al 2008a).
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9 10 **Discussion**

11 The use of benzoic and sorbic acid has more or less followed the rules laid down in the EU directive
12 (EU 1995) and in the Positive list (Fødevarestyrelsen 2008) through the years from 2001 to 2006
13 with only a few declaration faults and a few faults of using too much benzoic and sorbic acid at the
14 same time. They are both widely used in many food groups and from table II, III and IV it can be
15 seen that the extent to which these preservatives have been used has not changed over the years.
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22 To take into account brand loyalty (Leclercq et al. 2003), the present authors have found it relevant
23 as in the three earlier publications about food additive intake (Leth et al. 2007, 2008a and 2008b) to
24 carry out a high-intake calculation by only averaging samples with a content of benzoic acid or
25 sorbic acid respectively. However, it could also be argued that the average for all samples is a more
26 reasonable figure to put into the intake calculation, so that extremes do not dominate. Therefore,
27 this calculation has also been carried out, and the results for both calculations are shown in figure 1-
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36 The intake of benzoic acid as seen from figure 1 and 2 is very high. For the high intake calculation
37 about 10% of both men and women have a life-long intake of benzoic acid over the ADI value of 0-
38 5 mg/kg bw/day, and also with the average of all samples more than 5% of the people will have an
39 intake higher than the ADI value for men up to the age group 25-34 and for women up to 19-24
40 years old. The children in the age group 4-6 years old can have an intake more than three times the
41 ADI value of 5 mg. The maximum intakes calculated are 28 mg/kg bw/day for a boy in the age
42 group 7-10 years old and 31 mg/kg bw/day for a girl in the same age group.
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50 Also the intake of sorbic acid as seen from figure 3 and 4 is very high. More than 10% of the men
51 and more than 5% of the women will have a life-long intake over the ADI value of 0-25 mg/kg
52 bw/day with the high intake calculation, and children in the age group 4-6 years old have an intake
53 more than three times over the ADI value with a maximum intake of 147 mg/kg bw day for a boy
54 and 112 mg/kg bw day for a girl in this age group. However, the ADI value is not exceeded, when
55 the calculation is based on average of all samples.
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5 From figure 5 and 6 can be seen that soft drinks, dressing, fat based salads, pickled herrings and
6 marmalade are responsible for the largest part of the intake of benzoic acid, and it is the same food
7 groups contributing both for the average intake and high intake calculation, although soft drinks are
8 more dominating for the high intake calculation. In all these food groups a very large part of the
9 foods have added benzoic acid (from 63% to 94%) as can be seen from table I, which means that
10 the high intake calculation is quite a realistic calculation. There will be many people living only on
11 foods with added benzoic acid and thus many people with a very high intake of benzoic acid, and
12 already with the average of all the samples the intake gets very high for benzoic acid.
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21 The situation is different for sorbic acid, as can be seen from figure 7 and 8. Bread (rye bread, buns,
22 toast bread, flûtes) is the dominating source for the intake of sorbic acid with some contribution
23 from dressing and soft drinks. This gets even more pronounced for the high intake calculation
24 where bread is totally dominating with its 68% of the intake. However, only seven out of 42
25 products in this group contained sorbic acid, which means that both the average of all the samples
26 and the average of samples with sorbic acid content will exaggerate the intake, and especially for
27 the high intake calculation, although people who live exclusively on the seven products will have a
28 very high sorbic acid intake, that is people who consume only pre-packed sliced bread and rye
29 bread, where a maximum of 2 g sorbic acid and sorbates per kg is allowed.
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38 Information from other investigations is sparse. In 2005 the intake of benzoic and sorbic acid was
39 estimated in Australia (Food Standards Australia New Zealand 2005). The average estimated intake
40 for benzoic and sorbic acid was in all cases much lower than the ADI values. However, in 2-5 year
41 old boys and girls the 95th percentile of the estimated intake gave values of 120-140% of the ADI
42 value for benzoic acid, but no problems with sorbic acid. In Brazil (Tfouni and Toledo 2002) the
43 average estimated intakes of benzoic and sorbic acid were far below the ADIs, although it was
44 recognized that heavy soft drinks consuming persons could exceed the ADI for benzoic acid.
45 English (MAFF 1995), Japanese (Ishiwata et al. 2001), Polish (Traczyk et al. 2003) and Slovakian
46 (Janeková et al. 2004) estimations of average intake all showed values below the ADIs for all
47 population groups, but the intake distribution was not calculated.
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57 JECFA has established an ADI value of 0-5 mg/kg bw for benzoic acid and 0-25 mg/kg bw for
58 sorbic acid (JECFA 1996). In this investigation are found intakes for benzoic acid far exceeding the
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4 ADI value. One concern could be hypersensitivity, but any evidence for hypersensitivity of benzoic
5 acid is sparse and it is assumed that only 1-2% of children react towards food additives at all
6 (Fødevarestyrelsen 2004). In the ADI value is built in a safety factor of 100, and the intake studies
7 reported in the literature (WHO 2000) show no ill effects of doses up to 500 mg/kg bw/day over
8 several years.
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13 14 15 **Conclusions**

16 There is no reason to believe that the high intakes of benzoic acid with about 10% of the subjects
17 exceeding the ADI value and sorbic acid with 5-10% of the subjects exceeding the ADI value found
18 in this investigation will have any ill health effects. However, since people realistically can eat food
19 giving an intake of benzoic acid over the ADI value of 0-5 mg/kg bw for their whole life, a
20 reduction of the maximum limits should be considered especially in the food groups of soft drinks,
21 dressing and salads, which will have the greatest effect on the intake of benzoic acid and of sliced
22 bread, which will have the greatest effect on the intake of sorbic acid.
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31 **Acknowledgement**

32 Dietician Karin Hess Ygil, National Food Institute, is thanked for preparing the data for the intake
33 calculations.
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Table I. Average content of benzoic and sorbic acid by food categories.

Foodstuffs	Benzoic acid			Sorbic acid		
	Average all	Average max* mg/kg	With content/ All samples	Average all	Average max* mg/kg	With content/ All samples
Cheese	11	13	18/22	24	213	3/22
Pudding, mousse, buttermilk with lemon	2	11	10/52	54	700	4/52
Margarine, shortenings, minarine	0	0	0/11	86	475	2/11
Marmalade, jelly, raisins	81	382	49/231	273	564	112/231
Tomato concentrate, tomato puree, pickles	47	502	5/53	47	620	4/53
Stewed fruit	108	377	6/21	367	643	12/21
Chocolate, bitter and milk	0	0	0/3	0	0	0/3
Sweets, liquorice, fruit gum, chocolate bars	14	83	23/133	69	241	38/133
Müsli, cake cream	0	0	0/3	0	0	0/3
Rye bread, buns, toast bread, flûtes	1	13	2/42	250	1500	7/42
Cream pastry, plain cake, swiss roll	2	37	10/197	506	1186	84/197
Fillet, tenderloin, ham	0	0	0/47	10	460	1/47
Liver paste, paté	22	260	6/60	334	716	28/60
Salami, sausages	0	19	1/164	10	522	3/164
Lobster, mussels, frozen shrimps	0	0	0/23	0	0	0/23
Boiled shrimps, shrimps in brine	577	932	13/21	23	480	1/21
Fried fish	0	0	0/10	11	37	3/10
Smoked herring, salmon and eel	0	0	0/34	0	0	0/34
Pickled herring, salted ripened herring	773	987	36/46	387	574	31/46
Danish caviar (roe, lump-sucker)	774	1032	15/20	425	603	12/17
Surimi	890	890	1/1	17	17	1/1
Tuna in water/oil, sardines in oil, kippers	153	438	14/40	38	760	2/40
Dressing, mayonnaise, remoulade	312	450	36/52	788	820	50/52
Chilisauce, tomato ketchup, grill sauce	3,3	10	3/9	0	0	0/9
Fat based salads and spreadings	478	520	114/124	627	670	116/124
Soft drinks without CO2 and with sugar	36	93	20/52	19	69	14/52
Soft drinks without CO2, light	50	83	12/20	45	65	14/20
Soft drinks with CO2 and with sugar	83	107	38/49	33	65	25/49
Soft drinks with CO2, light	81	96	47/56	42	68	34/68

*Average of samples with a content of benzoic or sorbic acid

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Table 2. Average content of benzoic and sorbic acid in sampled fat containing foods. Results of four investigations (2001 – 2006).

Year	Foodstuffs	Benzoic acid			Sorbic acid		
		Average all mg/kg	With content*	With content/ All samples	Average all mg/kg	With content*	With content/ All samples
2001	Dressing, mayonnaise, remoulade, bernaise sauce	268	423	19/30	830	859	29/30
	Fat based salads and spreadings	445	538	134/162	669	677	160/162
2003	Dressing, mayonnaise, remoulade, bernaise sauce	422	600	19/27	800	800	27/27
	Fat based salads and spreadings	563	600	150/160	691	700	158/160
2005	Dressing, mayonnaise, remoulade, bernaise sauce	240	370	24/37	776	820	35/37
	Fat based salads and spreadings	467	500	141/151	657	680	146/151
2006	Dressing, mayonnaise, remoulade, bernaise sauce	312	450	36/52	788	820	50/52
	Fat based salads and spreadings	478	520	114/124	627	670	116/124

*Average of samples with a content of benzoic or sorbic acid

Table 3. Average content of benzoic and sorbic acid in sampled marmalade. Results of three investigations (2001 – 2004).

Year	Foodstuffs	Benzoic acid			Sorbic acid		
		Average all	With content* mg/kg	With content/ All samples	Average all	With content* mg/kg	With content/ All samples
2001	Marmalade, jelly, raisins	93	412	28/124	300	590	63/124
2003	Marmalade, jelly, raisins	60	298	17/85	242	541	38/85
2004	Marmalade, jelly, raisins	95	525	4/22	245	490	11/22

*Average of samples with a content of benzoic or sorbic acid

Table 4. Average content of benzoic and sorbic acid in sampled stewed fruit. Results from three investigations (2001 – 2004).

Year	Foodstuffs	Benzoic acid			Sorbic acid		
		Average all mg/kg	With content*	With content/ All samples	Average all mg/kg	With content*	With content/ All samples
2001	Stewed fruit	150	400	3/8	459	613	6/8
2003	Stewed fruit	48	240	1/5	216	540	2/5
2004	Stewed fruit	209	410	2/8	369	738	4/8

*Average of samples with a content of benzoic or sorbic acid

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Table I. Average content of benzoic and sorbic acids by food categories.

Table II. Average content of benzoic and sorbic acid in sampled fat containing foods. Results of four investigations (2001 – 2006).

Table III. Average content of benzoic and sorbic acid in sampled marmalade. Results of three investigations (2001 – 2004).

Table IV. Average content of benzoic and sorbic acid in sampled stewed fruit. Results from three investigations (2001 – 2004).

Figure 1. Estimated median, 90th and 95th percentiles of dietary exposure to benzoic acid by age groups considering all food products (average intake calculation) and considering food products with detectable content of benzoic acid (high intake calculation). Men.

Figure 2. Estimated median, 90th and 95th percentiles of dietary exposure to benzoic acid by age groups considering all food products (average intake calculation) and considering food products with detectable content of benzoic acid (high intake calculation). Women.

Figure 3. Estimated median, 90th and 95th percentiles of dietary exposure to sorbic acid by age groups considering all food products (average intake calculation) and considering food products with detectable content of sorbic acid (high intake calculation). Men.

Figure 4. Estimated median, 90th and 95th percentiles of dietary exposure to sorbic acid by age groups considering all food products (average intake calculation) and considering food products with detectable content of sorbic acid (high intake calculation). Women.

Figure 5. Foodgroups contribution in % to intake of benzoic acid considering all sampled food products (average intake calculation).

Figure 6. Foodgroups contribution in % to intake of benzoic acid considering sampled food products with detectable content of benzoic acid (high intake calculation).

Figure 7. Foodgroups contribution in % to intake of sorbic acid considering all sampled food products (average intake calculation).

Figure 8. Foodgroups contribution in % to intake of sorbic acid considering sampled food products with detectable content of sorbic acid (high intake calculation).

Figure 1. Estimated median, 90th and 95th percentiles of dietary exposure to benzoic acid by age groups for all food products (average intake calculation) and for food products with detectable content of benzoic acid (high intake calculation). Men.

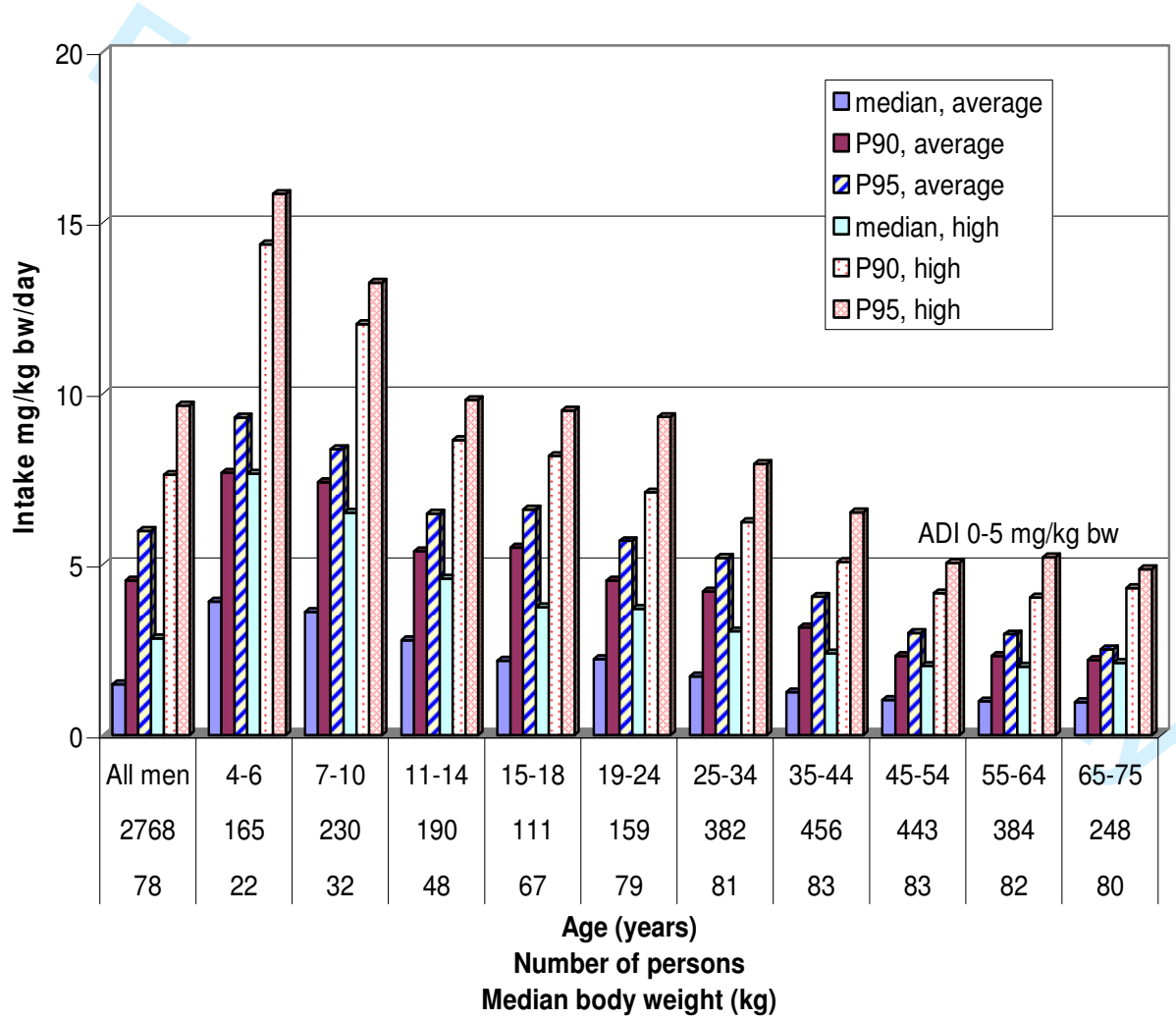


Figure 2. Estimated median, 90th and 95th percentiles of dietary exposure to benzoic acid by age groups for all food products (average intake calculation) and for food products with detectable content of benzoic acid (high intake calculation). Women.

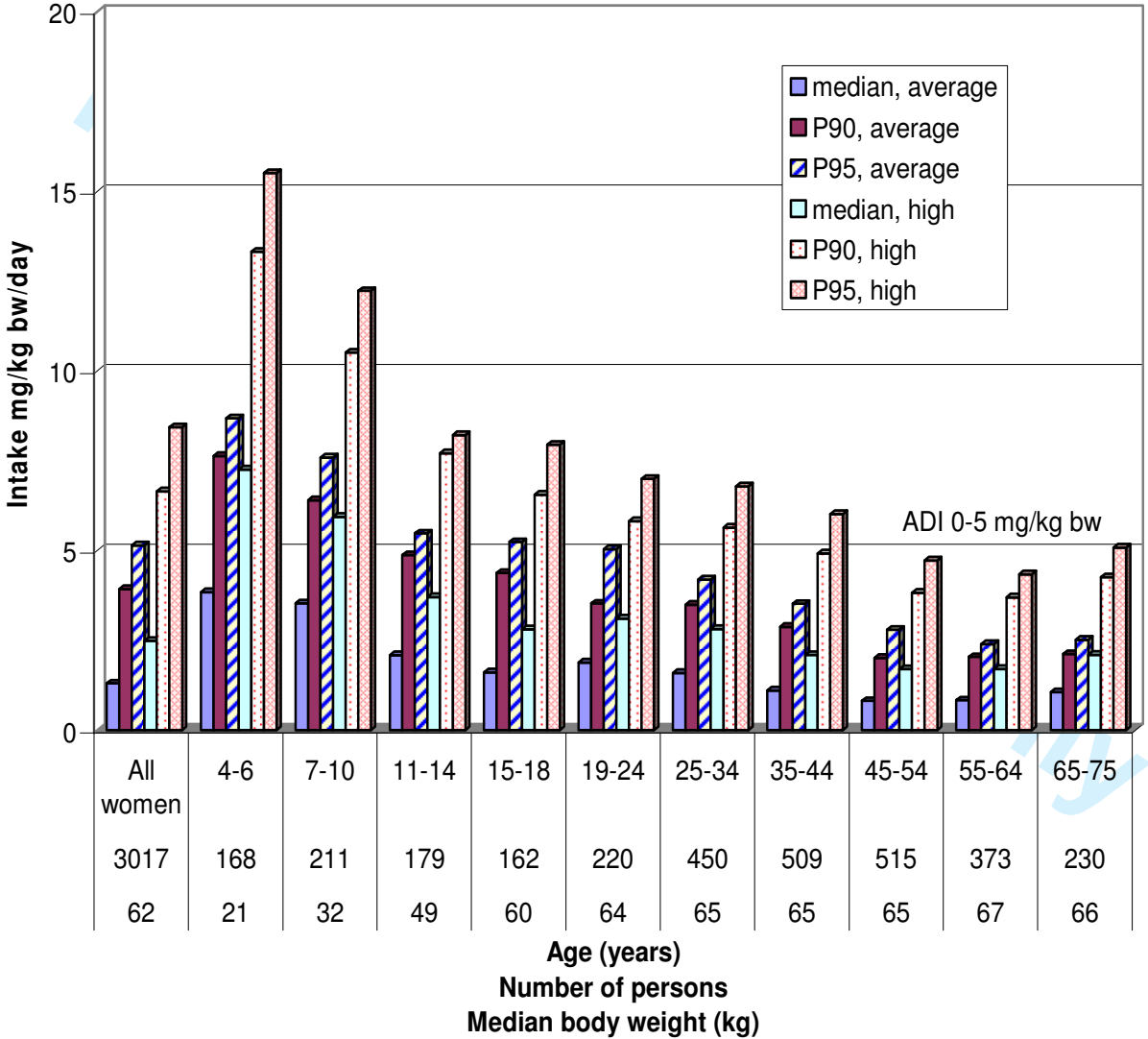


Figure 3. Intake of sorbic acid, men. All samples and samples with content

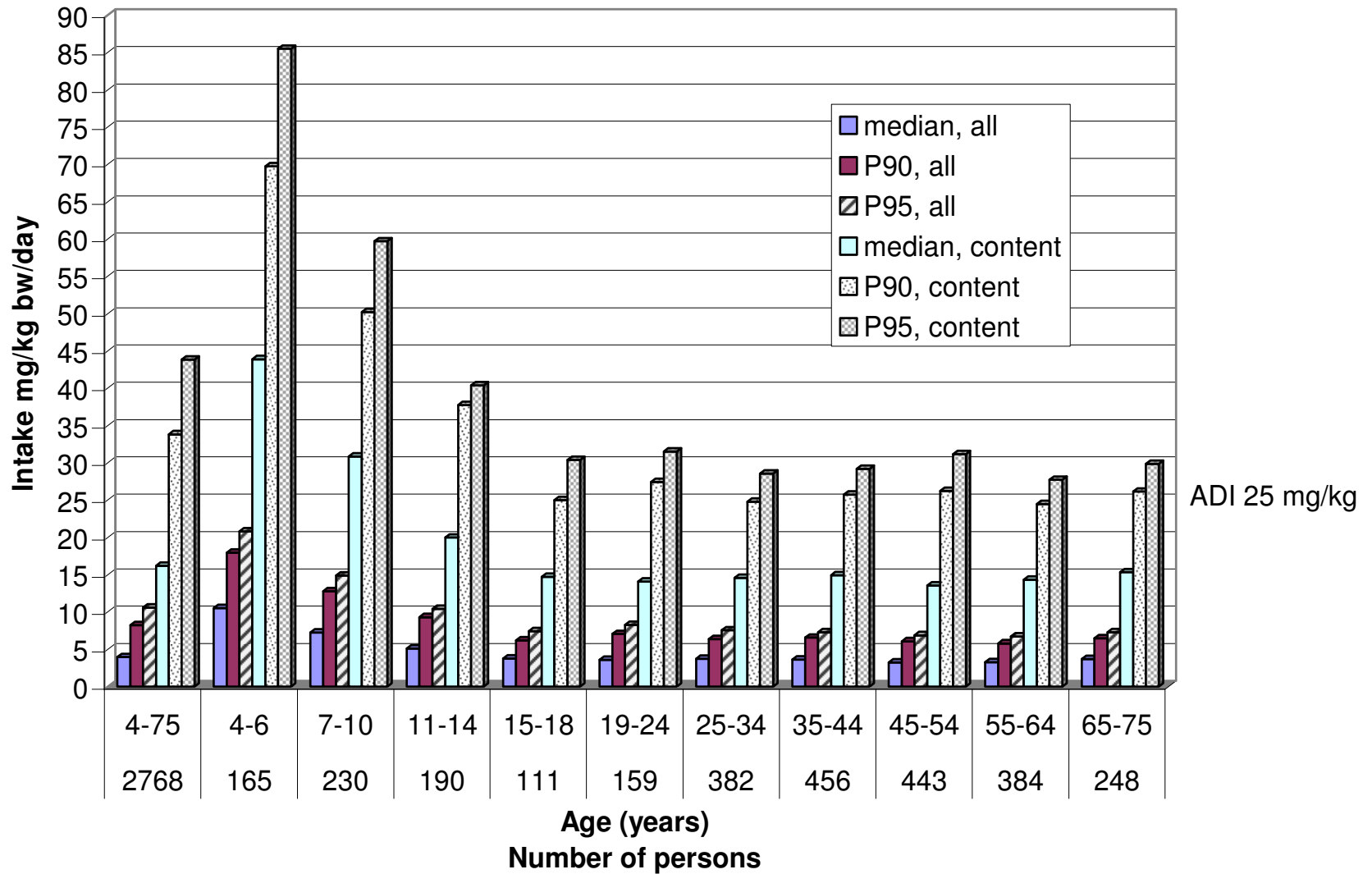


Figure 4. Estimated median, 90th and 95th percentiles of dietary exposure to sorbic acid by age groups for all food products (average intake calculation) and for food products with detectable content of sorbic acid (high intake calculation). Women.

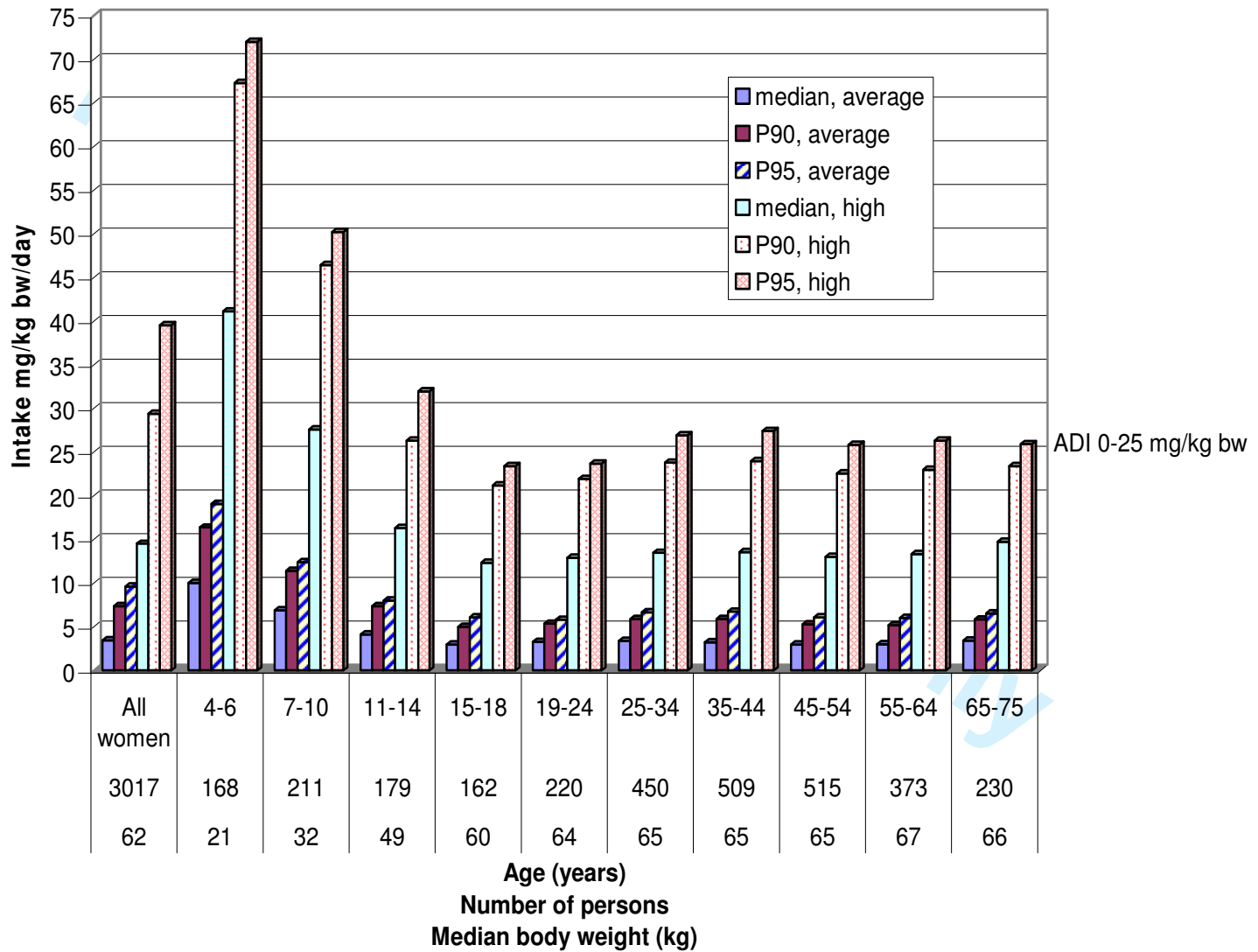
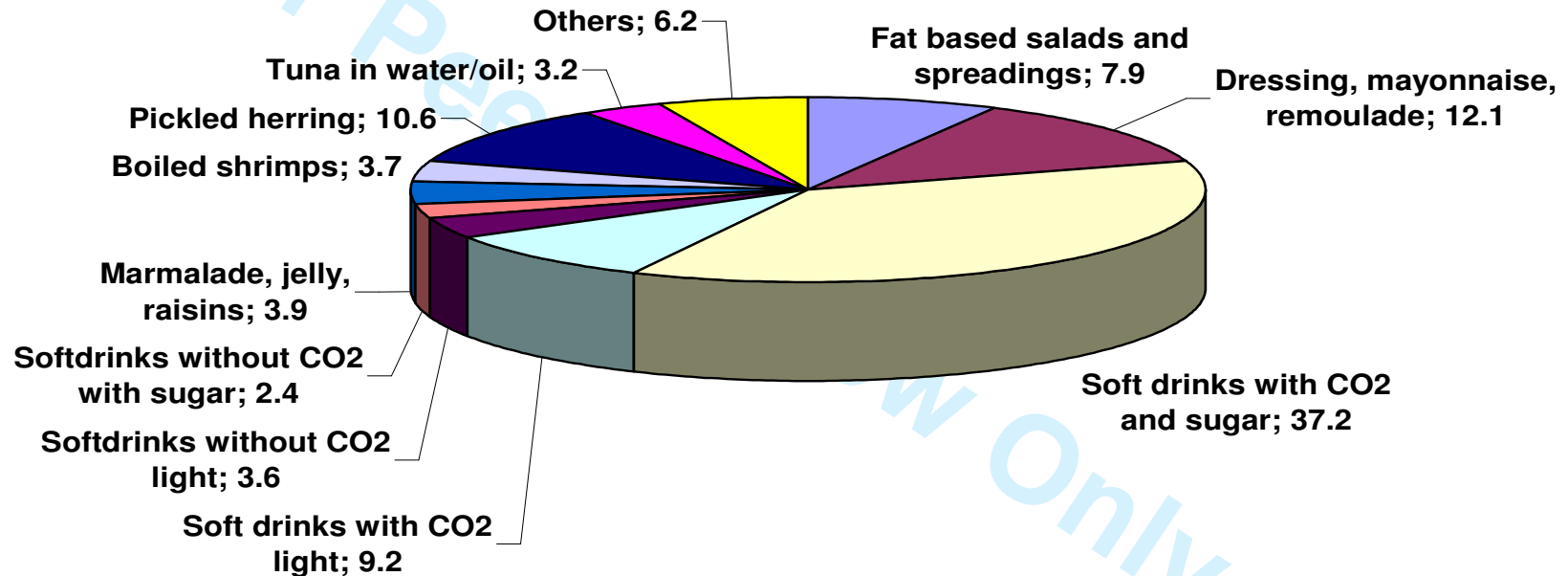


Figure 5. Benzoic acid, average all. Foodgroups contribution to intake in %



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Figure 6. Foodgroups contribution in % to intake of benzoic acid considering sampled food products with detectable content of benzoic acid (high intake calculation)

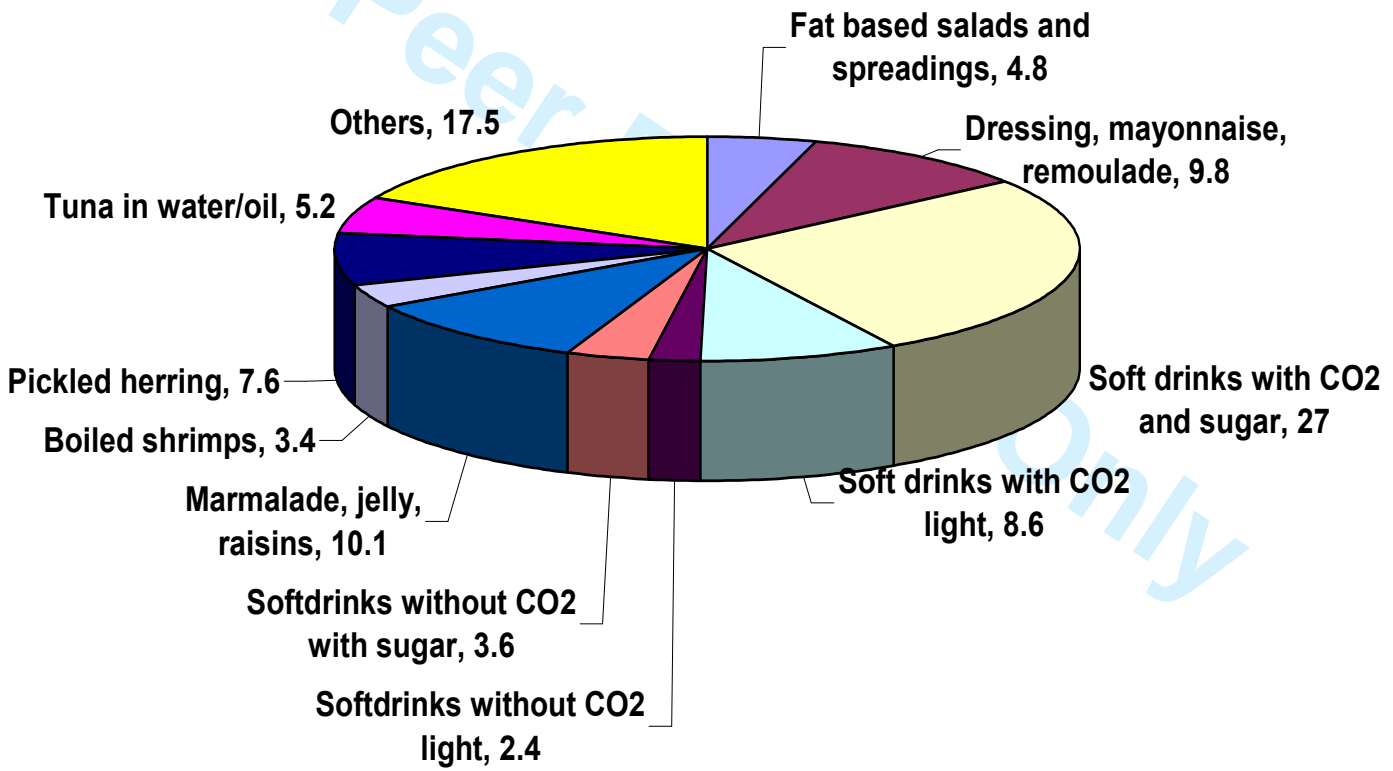


Figure 7. Foodgroups contribution in % to intake of sorbic acid considering all sampled food products (average intake calculation)

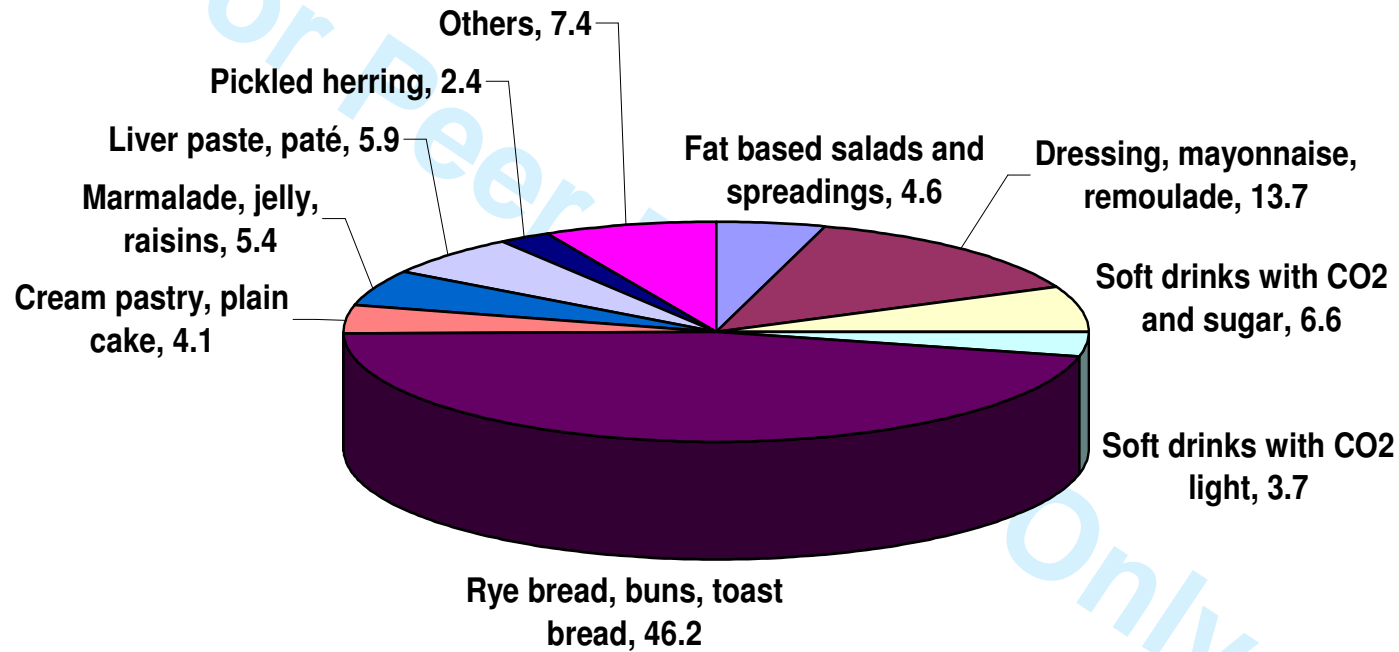


Figure 8. Foodgroups contribution in % to intake of sorbic acid considering sampled food products with detectable content of sorbic acid (high intake calculation)

