Fresh and processed mushrooms as a source of pro-healthy ingredients

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Mushroom production and consumption

- World production of mushrooms is about 6 million tons (FAOStat)
  - China 10 kg /person /year
  - Europe 1-4 kg /person /year
  - Poland 2 kg /person /year

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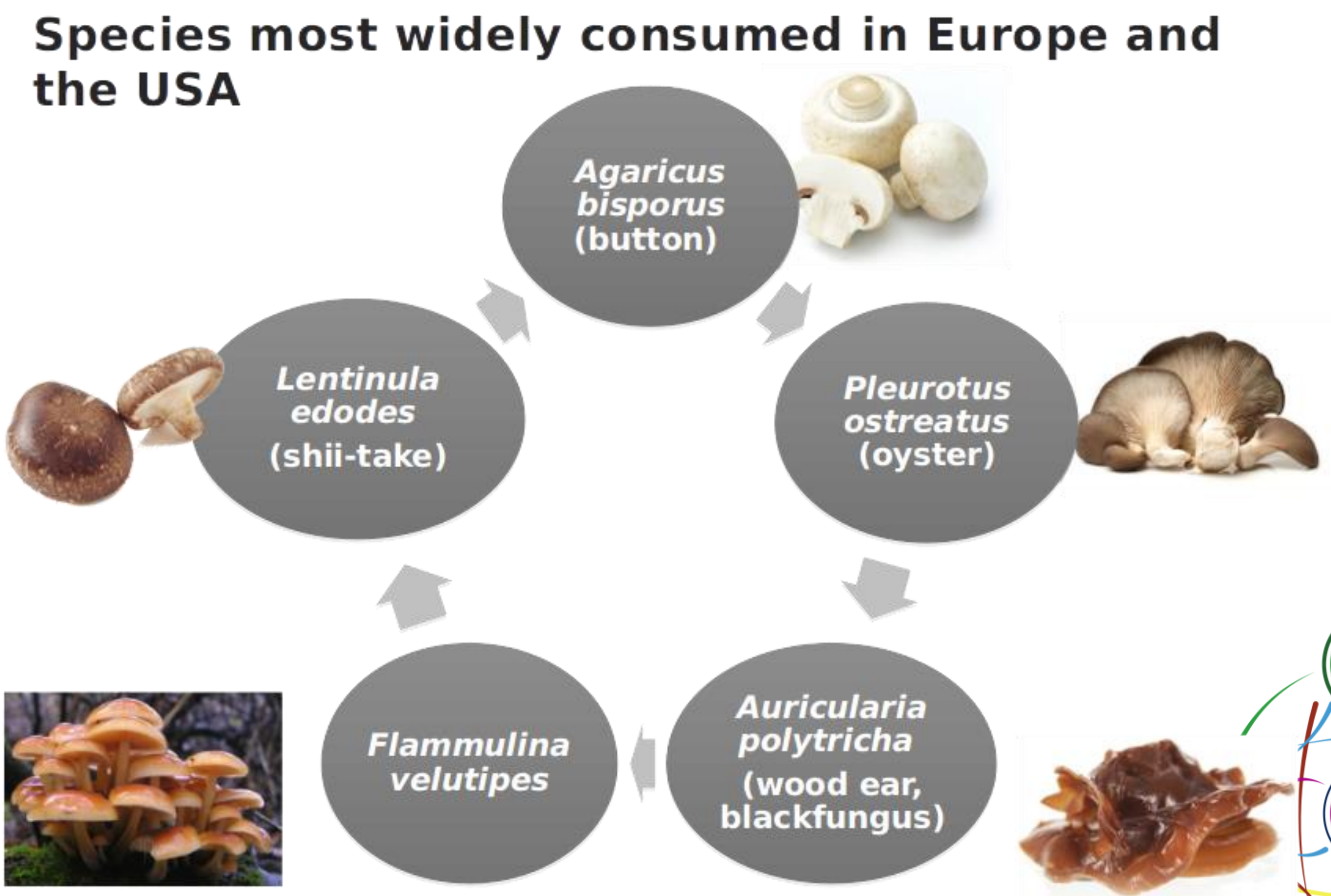
Species most widely consumed in Europe and the USA



Popular and less popular species of wild growing edible mushrooms in Poland

Lactarius deliciosus

Craterellus cornucopioides

Armillaria mellea

Leccinum spp.

Macrolepiota procera

Neoboletus erythropus

Chemical composition of edible mushrooms

Features of mushrooms

Plant origin

- The presence of a cell wall
- Phenolic compounds
- Polysaccharides - Beta-glucans
- Triterpenes
- Vitamin C

Animal origin

- Heterotroph organizm
- They are build from hyphae resembling animal tissue
- Contain animal cell organelles, e.g. nucleus, mitochondria, endoplasmic reticulum
- Lack of chlorophyll
- Chitin
- Synthesis of Vitamin D2
- Glycogen as backup material

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Factors influencing the chemical composition of mushrooms

1. Species

Auricularia auricula-judae

Boletus edulis

Sparassis crispa

Tuber melanosporum

Hericium erinaceus

Pseudohydnum gelatinosum

2. Type of substrate

<num ber>

Wild growing

Agaricus

Pleurotus

Grifola frondosa - Maitake

Cultivated

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Type of substrate …

<num

Wood

Auricularia auricula-judae

Armillaria mellea

Forest litter

Imleria badia

Lactarius deliciosus

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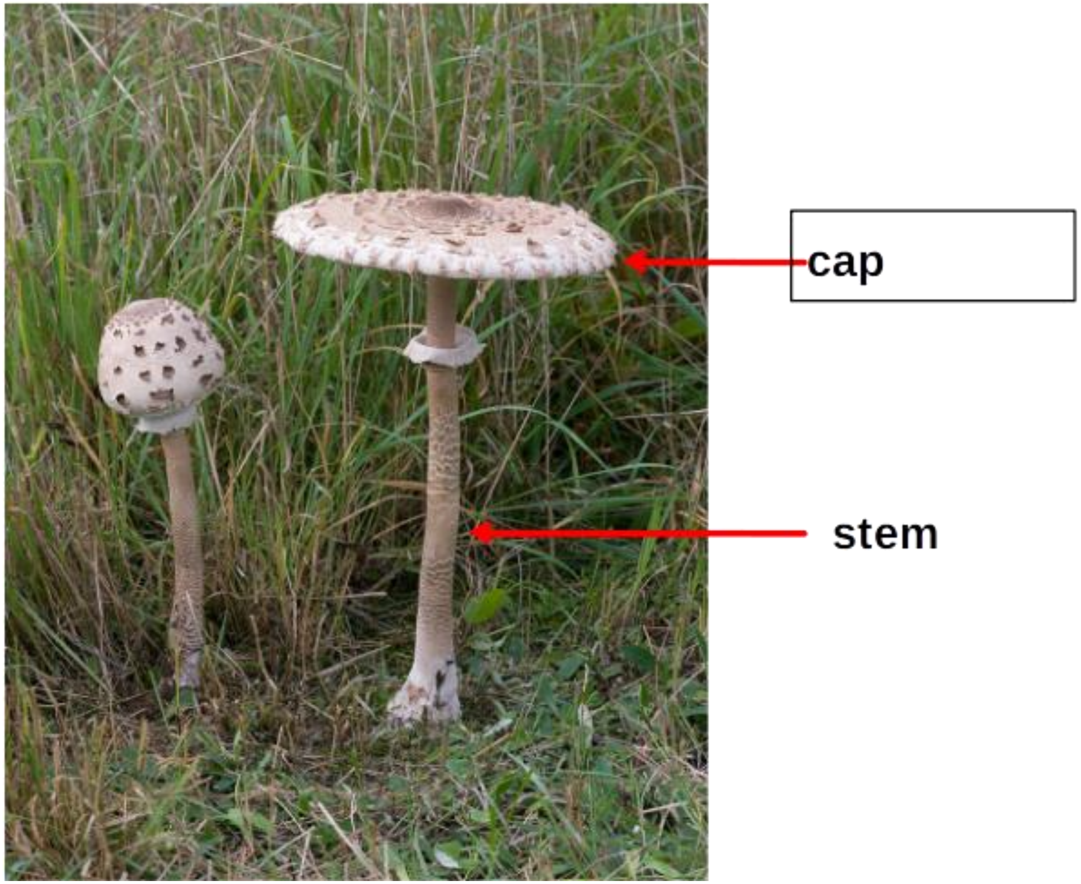
3. Part of the fruiting body

Macrolepiota procera



4. Age and size of the fruiting body

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Climatic conditions during growth

Nutritional and health-promoting components in mushrooms (in 100 g dm)

- Basic chemical components
  - protein (15-35 g)
- Vitamins
- B group: B1 (0.5-1.0 mg), B2 (2-5 mg), B3 (30-70 mg), folates (0.3-0.6 mg), vitamin D2 (22-110 µg), vitamin C (20-140 mg)
- Minerals (6-11g)
  - K ((2670–4730 mg), P (493–1390 mg), Cu (0.52–3.50 mg), Mg (20–200 mg), Zn (4.70–9.20 mg), Se (3.90–320 µg)
- Dietary fibre (TDF, SDF, IDF) (25-55 g)
  - β-glucans (0.2-0.6 g), chitin, chitosan
- Antioxidants
  - phenols, carotenoids, catalaze, ergothioneine, β-carotene
- Other
  - lectins, lovastatin, sesquiterpenes

Methods of analysis: HPLC, spectrophotometric, enzymatic

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Proximate composition of some edible wild-grown mushrooms of China (*mean* values; % of dry matter).

Species	Number of samples (*n*)	Carbohydrates	Crude fibre	Crude protein	Crude fat	Ash
B. aereus	1	34.0	17.0	26.9	2.1	8.5
B. edulis	1	30.6	15.3	28.7	4.1	9.2
B. speciosus	1	28.6	21.0	28.1	2.9	7.6
C. aureus	1	61.5	5.2	14.1	4.0	9.2
Lactarius deliciosus	1	25.0	36.3	20.2	2.5	7.5
Lactarius hatsudake	1	38.2	31.8	15.3	1.0	7.3
Lactarius volemus	1	15.0	40.0	17.6	6.7	13.3
L. crocipodium	1	12.8	37.9	29.3	1.0	5.8
Lentinula edodes	1	30.2	39.4	17.1	1.9	4.3
R. virescens	1	13.4	32.8	28.3	1.5	11.9
S. aspratus	1	64.6	5.1	12.0	2.8	10.4
T. matsutake	3	36.7	29.1	14.3	5.0	8.9

B. – Boletus, C. – Craterellus, L.- Leccinellum, R. – Russula, S.- Sarcodon, T. - Tricholoma

X.-M. Wang et al. / Food Chemistry 151 (2014) 279–285

Table 1. Recent data on dry matter (g kg^{-1}), proximate composition (g kg^{-1} DM) and energy (kcal kg^{-1} FM) in some wild-growing mu

Species	Dry matter	Crude protein	Lipids	Ash	Carbohydrates	Energy
Agaricus campestris	118.3	185.7	1.1	231.6	581.6	364
Armillaria mellea	117.3	163.8	55.6	67.8	712.8	470
	—	172.5	65.6	123.4	638.5	—
Boletus aereus	83.5	178.6	4.4	88.7	728.3	306
B. armeniacus	285.0	182.5	15.6	120.9	681.0	1053
B. edulis	108.5	210.7	24.5	55.3	709.5	423
B. erythropus	116.4	209.2	7.5	259.0	524.3	349
B. reticulatus	89.0	225.7	25.5	197.2	551.6	297
	—	279.0	31.4	166.2	523.4	—
Calocybe gambosa	90.8	154.6	8.3	138.9	698.2	317
Calvatia utriformis	220.0	203.7	19.0	178.1	599.2	744
Cantharellus cibarius	—	357.9	14.7	64.2	563.2	—
Clitocybe odora	115.1	173.3	24.6	95.5	706.6	431
Coprinus comatus	148.1	156.7	11.3	128.5	703.5	525
	—	294.7	54.2	158.8	492.3	—
Fistulina hepatica	83.3	500.9	18.9	164.0	316.2	286
Flammulina velutipes	93.2	178.9	18.4	94.2	708.5	346
Laccaria laccata	117.5	627.8	37.6	206.9	127.7	345
Lactarius deliciosus	—	202.0	80.2	71.5	646.3	—
L. salmonicolor	122.8	372.8	20.3	232.8	374.1	389
	—	135.3	10.9	61.6	792.2	—
Lycoperdon echinatum	147.6	235.2	12.2	94.3	658.3	544
Pleurotus ostreatus	—	132.3	35.8	80.8	751.1	—
Russula cyanoxantha	155.6	168.0	15.2	70.3	746.5	590
R. delica	133.1	505.9	9.1	229.3	255.7	416
R. olivacea	154.2	168.4	19.9	377.8	433.9	399
Suillus mediterraneensis	88.0	243.2	26.1	276.4	454.3	266
S. variegatus	92.3	175.7	33.1	153.6	637.6	328
Tricholoma imbricatum	175.8	504.5	18.8	64.5	412.2	674

Kalac. P. J Sci Food Agric 2013; 93: 209–218

Table 2. Recent data on dry matter (g kg^{-1}), proximate composition (g kg^{-1} DM) and energy (kcal kg^{-1} FM) in some cultivated mushr

Species	Dry matter	Crude protein	Lipids	Ash	Carbohydrates	Energy
garicus bisporus						
White	87.3	140.8	21.8	97.4	740.0	325
Brown	83.6	154.3	16.7	113.6	715.4	303
Unspecified	—	264.9	25.3	87.8	622.0	—
Unspecified	97.0	363.0	8.0	120.0	509.0	—
Agaricus brasiliensis	—	267.4	26.2	68.1	638.3	—
Flammulina velutipes	121.3	38.7	28.9	72.5	859.9	467
	—	266.5	92.3	75.1	566.1	—
ypsizigus marmoreus						
Normal strain	—	196.0	40.9	77.5	685.6	—
White strain	—	210.6	56.2	82.6	650.6	—
Lentinula edodes	202.2	44.0	17.3	67.3	871.4	772
	—	204.6	63.4	52.7	679.3	—
Pleurotus ostreatus	108.3	70.2	14.0	57.2	858.6	416
	—	238.5	21.6	75.9	664.0	—
	100.0	416.0	5.0	60.0	519.0	—
	88.0	166.9	54.5	67.0	711.6	—
P. eryngii	110.0	110.0	14.5	61.8	813.7	421
	—	221.5	15.7	57.6	705.2	—
P. sajor-caju	100.0	374.0	10.0	63.0	553.0	

Kalac. P. J Sci Food Agric 2013; 93: 209–218

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Essential amino acid composition in edible wild-grown mushrooms of China (mean values; mg kg^{-1} of dry matter).

Species	Number of samples (*n*)	Lys	Thr	Val	Ile	Leu	Met	Try	Phe	EAA
B. aereus	1	1040	1670	2560	3100	2350	440	–	1290	12450
B. eduli	1	990	2110	2750	2030	2470	750	–	1700	12800
B. speciosus	1	1200	2120	3730	4190	3500	690	830	1860	18120
Cortinarius rufo-olivaceus	1	16200	13900	36800	8300	10700	10400	–	9200	105500
C. aureus	1	4441	9230	6794	5054	7014	2813	–	4240	39586
L. delieiosus	1	960	930	1300	1350	2240	360	330	880	8350
L. hatsudake	1	750	890	1040	1620	2480	320	290	800	8190
Lactarius hygrophroides	1	21348	10227	12284	9787	13563	6676	12328	4561	90774
L. volemus	1	500	820	990	1490	2060	160	150	750	6920
L. crocipodium	1	1580	1750	2840	1080	1930	580	–	1240	11000
R. virescens	1	850	1350	1310	1360	1660	890	400	1440	9260
S. aspratus	1	4602	8479	4787	4187	5780	1476	–	3913	33224
Collybia albuminosa	1	13651	19889	12748	10231	19048	5900	–	10704	92170

B. – Boletus, C. – Craterellus, L.- Lactarius, R. – Russula, S.- Sarcodon

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Bioactive compounds

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Mushroom Species	Bio active Molecules	Medicinal Properties
Agaricus bisporous	Lectins	Enhance insulin secretion, anti-aging property.
Auricularia auricula	Acidic Polysaccharides	Anti-tumour activities, lowers cholesterol, triglycerides, and lipid levels; decrease blood glucose, beneficial in coronary heart disease, immune tonic
Cordyceps sinensis	Cordycepin	Cure lung infections, hypoglycemic activity, cellular health properties, anti-depressant activity.
Flammulina velutipes	Polysaccharide, flammulin, FVP (*Flammulina* polysaccharide protein), peptide glycans, prolamin (active sugar protein), Proflamin (glycoprotein)	Antioxidant, anti-cancer activity, anti-ageing property; immuno-modulatory, anti-viral action.
Ganoderma lucidum	Polysaccharides, triterpenoids, germanium, nucleotides and nucleosides, Ganoderic acid, Beta-glucan,	Augments immune system, liver protection, antibiotic properties, inhibits cholesterol synthesis; immunomodulatory, anti-cancerous properties.
Grifola frondosa	Polysaccharide, Lectins	Increases insulin secretion, decrease blood glucose, improves ovulation.
Lentinula edodes	Eritadenine, Lentinan	Lower cholesterol, anti-cancer agent.
P. florida		anti-hyperglycaemic; anti-hypercholesterolemia effect
P.sajor-caju	Lovastatin polysaccharide	Lower cholesterol, prevents cardiovascular disorders.
Trametes versicolor	Polysaccharide-K (Kresin), Coriolon and glycoproteins	Decrease immune system depression, prevents cancer, inhibits growth of *Candida albicans*, anti-viral activity by inhibiting the replication of HIV, liver protective functions.
Volvariella volvacea	Glycoproteins	Cardio-protective, lowers blood pressure.
Hericium erinaceus	Hericenones and erinacines	Neuritogenic effects

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Mushroom	Type of polysaccharides	Health benefits
Agaricus bisporus	Heteropolysaccharides	Activation of macrophages
Agaricus bitorquis	Homopolysaccharides	Activation of natural killer cells
Agaricus blazei	Glucan-protein complex	Activation of T lymphocytes
Auricularia auricula-judae	Homopolysaccharides	Anti-viral activity
Boletus erythropus	Homopolysaccharides	Antimicrobial activity
Calocybe indica	Homopolysaccharides	regulate lipogenesis
Ganoderma lucidum	Heteropolysaccharides	Induction of apoptosis
Geastrum saccatum	Glucan-protein complex	Treatment in stomach cancer
Grifola frondosa	Heteroploysaccharides Grifloan	Antitumor activity
Lentinus edodes	Heteropolysaccharides Lentinan	Antitumor activity
Phellinus linteus	Homopolysaccharides	Increase production of interleukin
Pleurotus eryngii	Homopolysaccharides	Antiproliferative effect
P. florida	Homopolysaccharides	Inhibit tumoral cell to cell adhesion
P. ostreatus	Homopolysaccharides	Increase gastrointestinal motility
Poria cocos	β-glucans type polysaccharides	Treatment of colon cancer
Polyporus rhinocerus	β-glucans type polysaccharides	Treatment of colon cancer
Schizophyllum commune	Homopolysaccharides Schizophyllan	Antitumor activity
Sparassis crispa	Homopolysaccharides	Lipid peroxidation inhibition
Termitomyces eurhizus	Homopolysaccharides	Anti-aging effects
T. microcarpus	Homopolysaccharides	Hepatoprotectiveactivity

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Type of terpenes	Mushroom species	Compound	Activity
Sesquiterpinoids	*P. cornucopiae*	Pleurospiroketal	Cytotoxic
	F. velutipes	Enokipodin J	Cytotoxic
		2,5-Cuparadiene-1,4-dione	Antioxidant Antibacterial Cytotoxic Antioxidant Antibacterial
	F. velutipes	Flammulinolide	Cytotoxic Antibacterial
	F. velutipes	Enokipodin	Antimicrobial
	L. subpiperatus	Lactarolide A	Promotional NA
Diterpenoids	*P. eryngii*	Eryngiolide A	Cytotoxic
	H.erinaceum	Erinacine A	Antibacterial
	Tricholoma sp.	Tricholomalide A Tricholomalide B Tricholomalide C	Cytotoxic
Triterpenoids	*G. lucidum*	Methyl ganoderate A acetonide *n*-Butyl ganoderate H Methyl ganoderate A Ganoderic acid B Ganoderic acid E Ganolucidic acid A Ganodermadiol Ganoderic acid Y Ganoderiol F Lucidumol B Ganodermanondiol Ganodermanontriol Lucidadiol	Anticholinesterase
	G. lucidum	Lucidenic acid N Lucidenic acid A	Anti-invasive
	G. lucidum	*n*-Butyl lucidenate N *n*-Butyl lucidenate A	Effect of on adipocyte differentiation in 3T3-L1 cells
	G. lucidum	Ganoderic acid Sz	Anticomplement Ganoderic acid C1
	G. amboinenese	Ganodermacetal Methyl ganoderate C	Toxic activity against brine shrimp larvae
	G. lucidum	Ganoderic acid DM	Cytotoxic
	G. amboinenese	Ganoderic acid X	Cytotoxic

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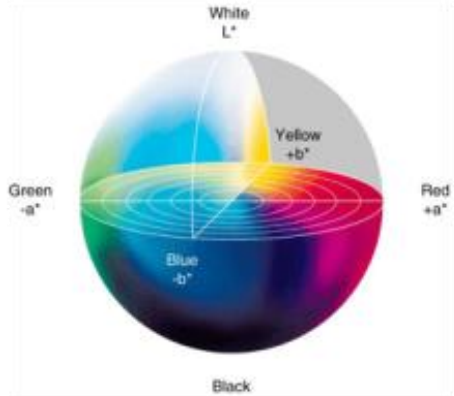
Other qualitative analysis of mushrooms

- **Color** - CIELAB



- **Texture** – texturometer

- **Organoleptic analysis**

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Storage of fresh mushrooms

- Edible mushrooms are highly perishable.
- The rapid deterioration in quality is mainly caused by:
  - high moisture content,
  - enzymatic activity,
  - microflora,
  - biological processes.

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Main changes during storage

- deterioration of sensory qualities, chiefly colour and texture
- fall in moisture content through evaporation
- cap opening
- stipe elongation
- decrease in nutritional value, including health-promoting components

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Method of mushroom processing

- A) Pre-treatment
  - Washing
  - Blanching
  - Vacuum soaking
- B) Processing
  - Freezing
  - Drying
  - Sterilization
  - Pickling
  - Lactic acid fermentation
  - Mushroom snacks e.g. mushroom jerky

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Pre-treatment

- The type of pre-treatment and the parameters of application should be selected according to the:
  - species of mushroom,
  - method of preservation,
  - storage time of the finished product.

Washing

- It is a fundamental operation in the pretreatment of mushrooms.
  - Promotes hygiene by removing mineral contamination and reducing microorganism counts.
  - Damages the cell membranes separating polyphenol oxidase from its substrates, which results in rapid darkening of the tissue.

- Used compounds: sodium metabisulphite, H_2O_2, EDTA, sodium erythorbate.

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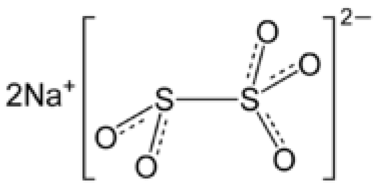
Sodium metabusulphite

- Is the most effective substance for maintaining light colour in mushrooms but whose residues may cause allergic reactions in consumers,
- Inhibit polyphenol oxidase activity and reactions with products remaining after enzymatic reactions, including o-quinones, which are converted to diphenols by sodium metabisulphite,
- The optimal concentration of sodium metabisulphite in solutions used prior to freezing A. bisporus is 4000 mg/dm^3 .



Blanching

- Water or water solutions containing compounds which prevent changes in mushroom colour
  - sodium metabisulphite (0,2-1%), H2O2, EDTA, citric acid, sodium erythorbate.
- Time: 1-3 min, tem. 85-95°C.
- Essential prior to freezing, canning and lactic acid fermentation.
- Mass losses 10-20%.

- Other methods
  - Steam.
  - Microwave - higher levels of dry matter and, ash and vitamins B1 and B2, fast inactivated of polyphenol oxidase.
  - High isostatic pressure - inactivate micro-organisms and enzymes without significantly affecting flavour or vitamins, adverse effect on mushroom colour.
  - Combination of microwave blanching and blanching in hot water - complete inactivation of polyphenol oxidase in a short time, good colour, considerable losses of mass and phenolic compounds.

Vacuum soaking

- Applied prior to blanching and preservation in order to reduce mass loss and increase yield.
- Time 15-20 min.

Laboratory

vacuum evaporator ->

Methods of processing mushrooms

- Canning
- Drying
- Freezing
- Salting
- Mushroom Jerky
- Fermenting
- Others

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Drying

- Usually hot air drying, initially at 40°C for 3 h, then at 60°C.
- Other methods:
  - freeze-drying,
  - freeze-drying combined with microwave drying,
  - freeze-drying combined with vacuum drying,
  - vacuum drying,
  - vacuum drying combined with microwave drying,
  - vacuum drying or freeze-drying in an adsorbent fluidized bed.

- Freeze-drying produces a high quality product, but is an expensive process.
- Microwave-vacuum drying resulted in a 70–90% decrease in the drying time and the dried products had better rehydration characteristics compared to convective air drying.

Canning - blanching is essential!!!

Pickled mushrooms

In glass jars.

In a sweet-salt-acidic brine solution containing acetic acid (1.5 to 4%), table salt and sugar.

Parameters: 85-87°C, 10-20 min.

Sterilised mushrooms

In glass jars or metal cans.

In salt or salt-acidic brine solutions containing approximately 2% sodium chloride and occasionally 0.05% citric acid, L-ascorbic acid, sodium metabisulphite

Parameters: 118-121°C, 10-20 min.

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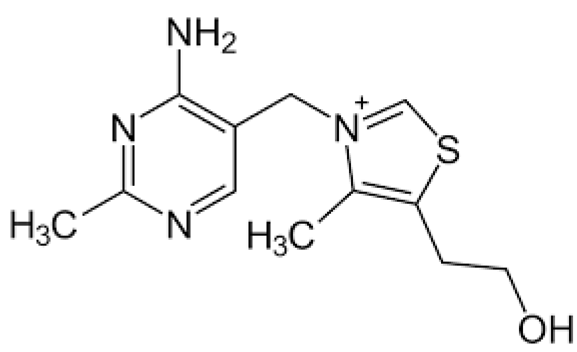
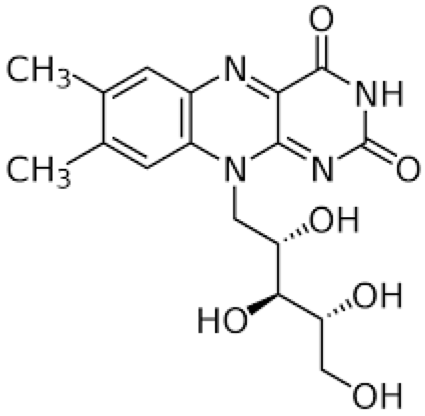
Method of determining vitamin B1 and B2 using HPLC methods





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Vitamin B1 and B2

- All sample vessels must be made of a material that is impervious to UV rays (eg. brown glass) or wrapped in aluminum foil, windows must be covered, vitamin B2 is sensitive to light.
- Two days analysis.

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Vitamin B1 and B2

- Metodology
  - PN-EN 14122:2004/AC:2006. Foodstuffs – Determination of vitamin B1 by HPLC.
  - PN-EN 14152:2004/AC:2006. Foodstuffs – Determination of vitamin B2 by HPLC.
- Main equipment
  - Sample incubator (temp. 121°C)
  - SPE Baker system
  - HPLC system: fluorescence detector, degasser, pump, columns thermostat, autosampler
  - HPLC column: Bionacom Velocity C18 PLMX (4.6 mm×250 mm, 5 μm) (Bionacom LTD, UK) with precolumn
  - SPE polypropylene column Chromabond (200 mg/3ml)
- Main reagents
  - 0.1n HCl, 2.5 M sodium acetate, taka-diastase, 15% NaOH, 0.005 M ammonium acetate, methanol, pure water (HPLC purity), potassium hexacyanoferrate(III) ($C_6FeK_3N_6$), 17% H_3PO_4, thiamine hydrochloride (purity ≥99%), riboflavin (purity ≥98%).

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Vitamin B1 and B2

- FIRST DAY
  - 1. In a beaker with a capacity of about 20 ml, weigh out 0.5 g of freeze-dried mushrooms and transfer (pour) into a conical flask with a capacity of 200-250 ml, add 60 ml of 0.1n HCl (use cylinder).
  - 2. Place the conical flasks in the sample incubator for 1 h, temperature 121°C (stir from time to time).
  - 3. Cool samples to a temperature < 50°C (place in a bowl with cold water).
  - 4. Adjust samples pH to 4.0-4.5 with 2.5 M sodium acetate.
  - 5. Add 5 ml of taka-diastase solution in 2.5 M sodium acetate (6%) and leave for 16-18 h in a sample incubator at 37°C, cover with aluminum foil.

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Vitamin B1 and B2

- THE SECOND DAY
  - 1. Place the conical flasks with the sample in a boiling water bath for 5 minutes to inactivate the enzyme, then immediately cool to temp. <30°C (a bowl with cold water).
  - 2. Adjust samples pH to 5.5-6.0 with 2.5 M sodium acetate.
  - 3. Quantitatively transfer the samples to a 100 ml volumetric flask using pure HPLC water, make up to the mark.
  - 4. Centrifuge samples for 15 minutes, 5000 rpm
  - 5. Filter supernatant through a filter paper into a conical flask (100 ml volume).

Vitamin B1 and B2

- THE SECOND DAY
  - 6. Thiochrome reaction:
    - a. Reaction mixture: take 2 ml of Fe3+ solution into a 50 ml volumetric flask and make up to the tap with 15% NaOH.
    - b. Collect 2 ml of the filtrate (point 5) into a 12 ml plastic tube.
    - c. Add 2 ml of Fe^{3+} solution in 15% NaOH (point 6a), shake 10 s (vortex), leave for 2 minutes.
    - d. Immediately adjust pH sample to 6.8-7.0 with 17% H_3PO_4.
  - 7. Transfer samples to centrifuge vessels and centrifuge for 5 minutes, 15,000 rpm.

Vitamin B1 and B2

- THE SECOND DAY
  - 8. Purification on an SPE - column SPE C18, 200 mg, 3 ml
    a. SPE column activation by 2.5 ml with methanol.
    b. Elution of methanol form SPE column with 2.5 ml of ammonium acetate (0.005 M).
    c. Placing supernatant (p. 7) on the SPE column (Note - the sample does not fit completely !).
    d. Washing the sample with 2.5 ml of ammonium acetate (0.005 M).
    e. Elution of the sample with 3 ml of mobile phase (60% of methanol + 40% of 0.005 M ammonium acetate) to pure 12 ml plastic tube.

Important: the SPE column can be used about 10 times, each time it must be cleaned in accordance with point 8a-8b.

HPLC analysis

- Standards:
  - thiamine hydrochloride in hydrochloric (vitamin B1),
  - (-)- riboflavin in acetic acid (vitamin B2).
- Eluent: water (HPLC purity) and acetonitrile
- Elution conditions:
  - Flow Rate: 0.9 mL/min
- Gradient elution: t=0 min water/acetonitrile ratio of 88:12; t=12 min water/acetonitrile ratio of 0:100.
- Column temperature: 22°C
- Wavelengths:
  - excitation 360 nm
  - emission 503 nm

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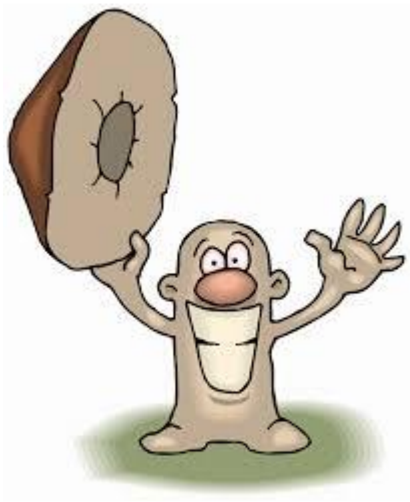
Standard curves (vitamin B1 and B2)

- Thiamine hydrochloride (purity ≥99%), concentration: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 (µg/1ml of sample)
- Riboflavin (purity ≥98%), concentration: 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 (µg/1ml of sample)



Thank you for your attention

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